Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2813796 C 2019/01/15

(11)(21) **2 813 796**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 2011/09/12
(87) Date publication PCT/PCT Publication Date: 2012/04/26
(45) Date de délivrance/Issue Date: 2019/01/15
(85) Entrée phase nationale/National Entry: 2013/04/05
(86) N° demande PCT/PCT Application No.: EP 2011/065771
(87) N° publication PCT/PCT Publication No.: 2012/052230
(30) Priorité/Priority: 2010/10/18 (US61/394,103)

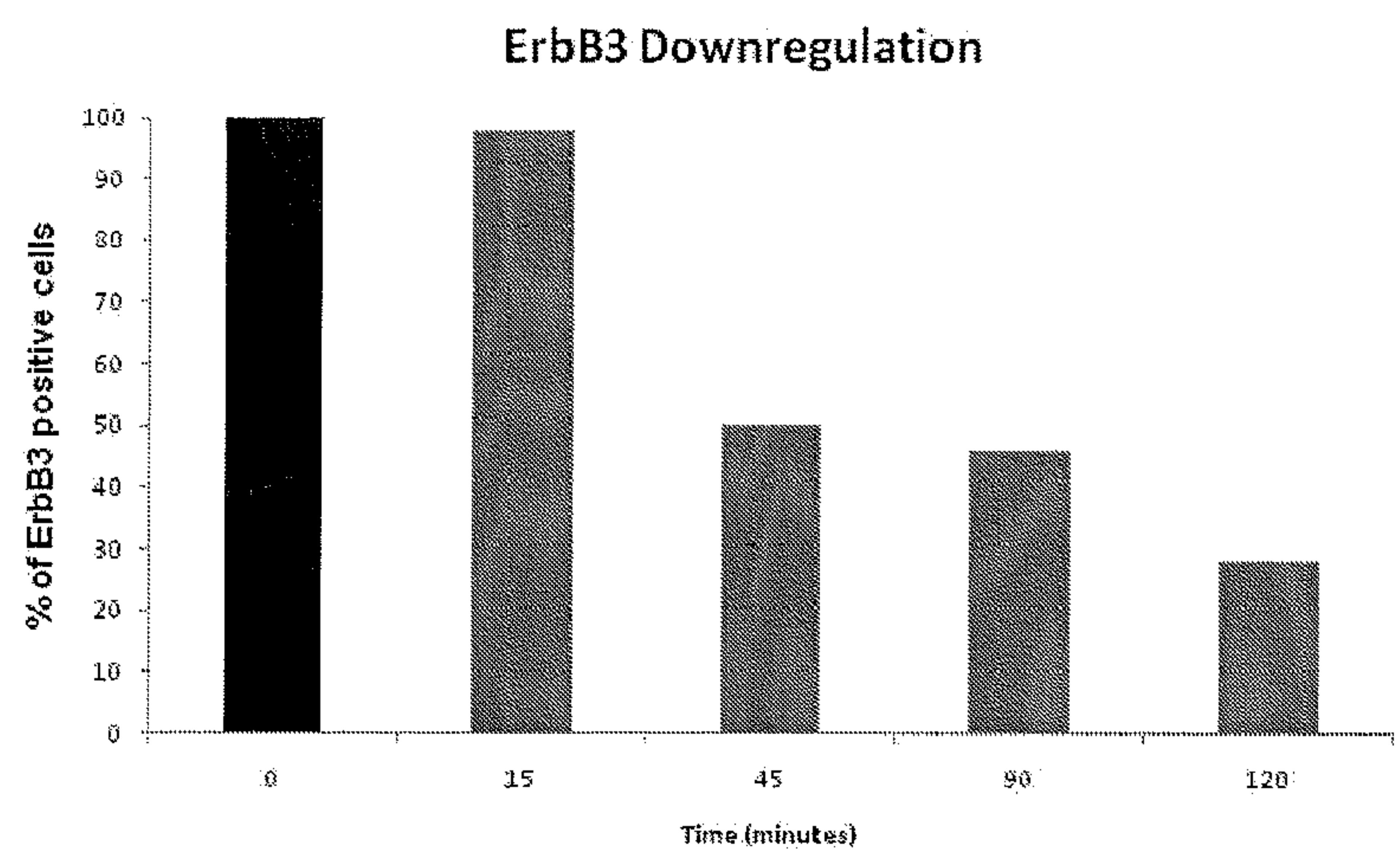
(51) Cl.Int./Int.Cl. *C07K 16/32* (2006.01), *A61K 39/395* (2006.01), *A61P 35/00* (2006.01)

(72) Inventeurs/Inventors:
MURARO, RAFFAELLA, IT;
IACOBELLI, STEFANO, IT;
TINARI, NICOLA, IT;
SALA, GIANLUCA, IT;
TRAINI, SARA, IT

(73) Propriétaire/Owner:
MEDIAPHARMA S.R.L., IT

(74) Agent: NORTON ROSE FULBRIGHT CANADA LLP/S.E.N.C.R.L., S.R.L.

(54) Titre : ANTICORPS SE LIANT A ERBB3
(54) Title: ERBB3 BINDING ANTIBODY



(57) Abrégé/Abstract:
The present invention relates to an antibody, particularly a monoclonal antibody, which binds to the ErbB3 receptor, compositions comprising such an antibody as well as methods using such an antibody.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 April 2012 (26.04.2012)

PCT

(10) International Publication Number
WO 2012/052230 A1

(51) International Patent Classification:
C07K 16/32 (2006.01) *A61P 35/00* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/EP2011/065771

(22) International Filing Date:
12 September 2011 (12.09.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/394,103 18 October 2010 (18.10.2010) US

(71) Applicant *(for all designated States except US)*: **MEDIAPHARMA S.R.L.** [IT/IT]; Centre of Excellence on Aging, Via Colle dell´Ara, I-66013 Chieti (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MURARO, Raffaella** [IT/IT]; Via della Lobbra, 2, I-00062 Bracciano (RM) (IT). **IACOBELLI, Stefano** [IT/IT]; Via Chiana, 48, I-00198 Roma (IT). **TINARI, Nicola** [IT/IT]; Via Chieti, 35, I-65015 Montesilvano (IT). **SALA, Gianluca** [IT/IT]; Via Emanuele Di Simone 4, I-65125 Pescara (IT). **TRAINI, Sara** [IT/IT]; Via Po, 75, I-63082 Castel di Lama (IT).

(74) Agents: **HEUBECK, Christian** et al.; Weickmann & Weickmann, Postfach 860 820, 81635 München (DE).

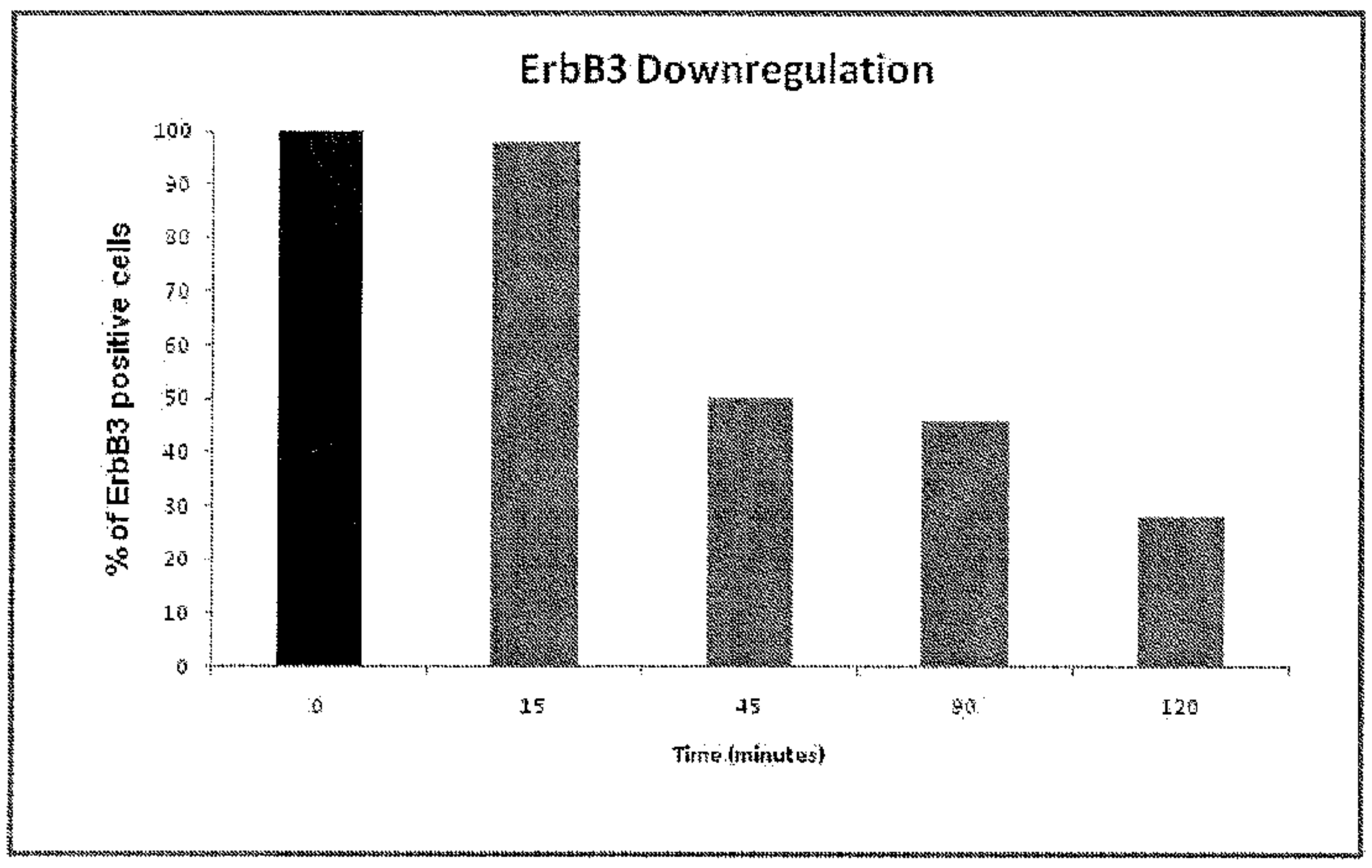
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) **Title**: ERBB3 BINDING ANTIBODY

Figure 1



(57) **Abstract**: The present invention relates to an antibody, particularly a monoclonal antibody, which binds to the ErbB3 receptor, compositions comprising such an antibody as well as methods using such an antibody.

WO 2012/052230 A1

WO 2012/052230 A1

Published:

— *with international search report (Art. 21(3))*

ErbB3 binding antibody

Description

The present invention relates to an antibody, particularly a monoclonal antibody, which binds to the ErbB3 receptor, wherein said binding reduces ErbB3 receptor mediated signal transduction, and compositions comprising such an antibody as well as methods using such an antibody.

Cancer is a disease characterized by uncontrolled proliferation of transformed cells that invade and destroy adjacent tissues, and may spread to distant anatomic sites through a process called metastasis[1-4]. In presence of metastatic disease, cancer may cause death in a variable period of time between a few months and some years[1-4].

The most used drugs for cancer treatment are cytotoxic chemotherapeutic agents (also called antiblastic agents or chemotherapeutic agents). These drugs act by damaging DNA or inhibiting cell proliferation. In this way, they kill all rapidly dividing cells, not only cancer cells, but also normal cells that are undergoing cell division. The lack of specificity of action of chemotherapeutic drugs on cancer cells is responsible for considerable toxicity following their administration. In the last decade, basic scientific research has significantly increased our knowledge about molecular mechanisms of cellular transformation and cell proliferation, leading to the development of "molecularly targeted" drugs or "targeted therapies"[5]. They refer to drugs that are specifically designed to act on cancer cells bearing particular molecular and/or functional abnormalities. However, also targeted therapies are associated with side effects, and in most cases they can block tumor growth only temporarily.

ErbB3 receptor, also known as HER-3, belongs to the epidermal growth factor receptor tyrosine kinase family (ErbB). This family of receptors consists of four members: ErbB1 (HER1), ErbB2 (HER2), ErbB3 (HER3) and

ErbB4 (HER4). Many studies have suggested a critical role for ErbB receptors in cell survival, proliferation and differentiation, as well as in malignant transformation[6-7]. The signal transduction mediated by tyrosine kinase receptors is complex and involves the interaction with two categories of ligands: epidermal growth factor (EGF) and EGF-like ligands (e.g. TGFα and amphiregulin), Neuregulin (NRG), also defined Heregulin (HRG) or Neu Differentiation Factor (NDF). Ligand binding to ErbB receptors induces the formation of receptor homo- and heterodimers and activation of the intrinsic kinase domain, resulting in phosphorylation on specific tyrosine residues within the cytoplasmic tail. These phosphorylated residues serve as docking sites for a range of proteins, the recruitment of which leads to the activation of intracellular signalling pathways. Generally, heterodimerization is preferred over homodimerization; ErbB2 is the preferred heterodimerization partner of the other ErbB receptors, including ErbB1 (activated by EGF or EGF-like ligands), and ErbB3 and ErbB4 (activated by neuregulin, NRG). The two major signaling pathways activated by ErbB receptors are Ras-Raf-MAPK and PI3K-AKT pathways.[8-10]

ErbB2 gene is amplified in 20 to 30% of breast cancers and is correlated with a poor prognosis. In the same way, ErbB3 receptor has also been shown to be overexpressed in breast cancer patients. High levels of expression of both ErbB2 and ErbB3 receptors are associated with an aggressive biology of tumor. In fact, upon NRG stimulation, ErbB2/ ErbB3 heterodimers deliver the most potent and long-lasting proliferative intracellular signal among the possible combinations of pairs of ErbB family members[8-11]. Several studies have suggested an important role of ErbB3 receptor in progression of many human tumor types, such as prostate cancer, melanoma, and gastric carcinoma.

All together, experimental and clinical data indicate that ErbB3 plays an essential role in tumor development and progression, suggesting that agents targeting ErbB3 could provide a novel and promising approach toward the treatment of some cancers.[12-24]

In spite of scientific progress and introduction into clinical practice of new chemotherapeutic agents and targeted therapies, cancer remains a disease difficult to cure, responsible for about 13% of deaths worldwide.[1-4]

Consequently, there is an urgent need to develop new antitumor therapies, more effective and possibly less toxic.

The inventor has found that specific ErbB3 inhibitors are able to induce tumor regression. In particular, the monoclonal antibody, MP-RM-1, has been used as an anti- ErbB3 inhibitor.

Thus, a first aspect of the present invention relates to an antibody or fragment thereof which binds to the ErbB3 receptor and which comprises

a) a heavy chain amino acid sequence as encoded by SEQ ID NO: **1** or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereto

and/or

b) a light chain amino acid sequence as encoded by SEQ ID NO: **2** or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereto.

<u>VH sequence (SEQ ID NO:1)</u>

gacgtgcagctggtggagtctgggggagacttagtgaagcctggagggtccctgaaactctcctgtgtagtctctggattcactttcagtac
ctatggcatgtcttgggttcgccagactccagacaggaggctggagtgggtcgcaaccattagtcatggtgacggttatacctactatcca
gacagtgtgaaggggcgattcaccatctccagagacaatgccaagaacaccctgcacctgcaaatgagcagtctgaagtctgagga
cacagccatgtattactgtgcaagacatggggattacgacgatgattactatgctatggactactggggtcaaggaacctcagtcaccgt
ctca

<u>VL sequence (SEQ ID NO:2)</u>

gatattgtgatgacccagtctccatcctccctgactgtgatagcaggagagaaggtcactatgagctgcaagtccagtcagagtctgttaa
acagtggaaatcaaaagaactacttgacctggtaccaacagaaaccagggcagcctcctaaactgttgatctactgggcatccactag
ggaatctggggtccctgatcgcttcacaggcagtggatctggaacagatttcactctcaccatcagcagtgtgcaggctgaagacctggc
agtttattactgtcagaatgaatatacttatccgctcacgttcggtgctgggaccaagctggagctgaaacggg

The term "antibody" as used herein includes "fragments" or "derivatives", which have at least one antigen binding site of the antibody and/or show the same biological activity.

Further, the antibody preferably comprises at least one heavy immunoglobulin chain and at least one light immunoglobulin chain. An immunoglobulin chain comprises a variable domain and optionally a constant domain. A variable domain may comprise complementary determining regions (CDRs), e.g. a CDR1, CDR2 and/or CDR3 region, and framework regions.

As used herein, "sequence identity" between two polypeptide sequences, indicates the percentage of amino acids that are identical between the sequences, preferably over the entire length of the amino acid sequences as encoded by SEQ ID NO: **1** and/or SEQ ID NO: **2.** Preferred polypeptide sequences of the invention have a sequence identity of at least 80%, more preferably 85%, even more preferably 90%, 93%, 95%, 96%, 97%, 98% or 99%.

According to another preferred embodiment, antibodies of the invention reduce ErbB3 receptor mediated signal transduction. Said reduction of ErbB3 receptor mediated signal transduction is preferably caused by a down-regulation of ErbB3. According to a further embodiment, down-regulation of ErbB3 is preferably achieved by decreasing levels of ErbB3 on the cell surface, i.e. preferably the antibody of the invention has the ability to decrease levels of ErbB3 on cell surfaces.

The antibody of the invention may have at least one antigen binding site, e.g. one or two antigen binding sites.

The antibodies of the invention bind preferably to the extracellular domain of ErbB3.

The antibody may be any antibody of natural and/or synthetic origin, e.g. an antibody of mammalian origin. Preferably, the constant domain -if present- is a human constant domain. The variable domain is preferably a mammalian variable domain, e.g. a humanized or a human variable domain.

Antibodies according to the invention may be polyclonal or monoclonal antibodies. Monoclonal antibodies are preferred. In particular antibodies of the present invention are preferably selected from the group consisting of recombinant antibodies, humanized or fully human antibodies, chimeric antibodies, multispecific antibodies, in particular bispecific antibodies, or fragments thereof.

Monoclonal antibodies may be produced by any suitable method such as that of Köhler and Milstein [25]or by recombinant DNA methods. Monoclonal antibodies may also be isolated from phage antibody libraries using techniques described in Clackson et al.[26]

Humanized forms of the antibodies may be generated according to the methods known in the art such as chimerization or CDR grafting. Alternative methods for the production of humanized antibodies are well known in the art and are described in, e.g., EP-A1 0 239 400 and WO 90/07861. Human antibodies can also be derived by in vitro methods. Suitable examples include but are not limited to phage display, yeast display, and the like.

According the present invention "chimeric antibody" relates to antibodies comprising polypeptides from different species, such as, for example, mouse and human. The production of chimeric antibodies is described, for example, in WO 89/09622.

Monospecific antibodies are antibodies that all have affinity for the same antigen. Multispecific antibodies are antibodies that have affinity for several antigens. A bispecific antibody has affinity for two different antigens.

The term antibody includes "fragments" or "derivatives", which have at least one antigen binding site of the antibody. According to a preferred embodiment the antibody or fragment thereof may be a Fab fragment, a Fab' fragment, a F(ab') fragment, a Fv fragment, a diabody, a ScFv, a small modular immunopharmaceutical (SMIP), an affibody, an avimer, a nanobody, a domain antibody and/or single chains.

"Avimer" relates to a multimeric binding protein or peptide engineered using, for example, in vitro exon shuffling and phage display. Multiple binding domains are linked, resulting in greater affinity and specificity compared to single epitope immunoglobin domains.

"Nanobody" or single domain antibody relates to an antibody fragment consisting of a single monomeric variable antibody domain.

"Affibody" molecules are small high affinity proteins being engineered to bind specifically to a large number of target proteins.

The antibody of the invention may be preferably of the IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE antibody-type. It will be appreciated that antibodies that are generated need not initially possess such an isotype but, rather the antibody as generated can possess any isotype and that the antibody can be isotype-switched.

The antibodies or antibody fragments of the invention are optionally deimmunized for therapeutic purposes.

In an especially preferred embodiment of the invention, the antibody is MP-RM-1, deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) on 15 October 2009 and designated DSM ACC3018.

According to a further preferred embodiment the antibody or fragment thereof may be produced from the hybridoma cell line DSM ACC3018 or a derivative thereof.
According to a preferred embodiment of the invention, the antibody or

fragment produced from the hybridoma cell line DSM ACC3018 or a derivative thereof is a humanized antibody or fragment thereof. Preferably, this humanized antibody comprises at least one of the sequences 3-14 of the present invention. According to an especially preferred embodiment, the antibody is selected from the group cMP-RM-1 #1, cMP-RM-1 #2, cMP-RM-1 #3, cMP-RM-1 #4, hMP-RM-1 #5, hMP-RM-1 #6, hMP-RM-1 #7, hMP-RM-1 #8, hMP-RM-1 #9, hMP-RM-1 #10, hMP-RM-1 #11, hMP-RM-1 #12, hMP-RM-1 #13, hMP-RM-1 #14, hMP-RM-1 #15, hMP-RM-1 #16, hMP-RM-1 #17, hMP-RM-1 #18, hMP-RM-1 #19, hMP-RM-1 #20 (c, chimeric antibody; h, humanized antibody).

An especially preferred embodiment relates to an antibody which is selected from the group cMP-RM-1 #4, hMP-RM-1 #14, hMP-RM-1 #17 or hMP-RM-1 #20.

Another preferred embodiment relates to the group of antibodies consisting of hMP-RM-1 #6, hMP-RM-1 #7, hMP-RM-1 #8, hMP-RM-1 #9, hMP-RM-1 #10, hMP-RM-1 #11, hMP-RM-1 #12, hMP-RM-1 #19 and hMP-RM-1 #20. A particularly preferred group of antibodies comprises the antibodies hMP-RM-1 #6, hMP-RM-1 #10 and hMP-RM-1 #20.

It will be apparent to those skilled in the art that the antibodies of the invention can be further coupled to other moieties for, e.g., drug targeting and imaging applications. Such coupling may be conducted chemically after expression of the antibody or antigen to site of attachment or the coupling product may be engineered into the antibody or antigen of the invention at the DNA level.

Thus, for diagnostic purposes, the antibody or antibody fragment of the invention may be labelled, i.e. coupled to a labelling group. Suitable labels include radioactive labels, fluorescent labels, suitable dye groups, enzyme labels, chromogenes, chemiluminescent groups, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter etc.

Those labelled antibodies or antibody fragments may be in particular used in immunohistochemistry assays or for molecular imaging *in vivo*.

For therapeutic purposes, the antibody or antibody fragment of the invention may be conjugated with a effector group, in particular a therapeutic effector group such as a radioactive group or a cytotoxic group.

Labelling groups or effector groups may be attached by spacer arms of various lengths to reduce potential steric hindrance.

According to another aspect, the present invention relates to a nucleic acid molecule encoding the antibody of the invention or fragment thereof or a nucleic acid capable of hybridizing thereto under stringent conditions. The nucleic acid molecule of the invention encoding the above-described antibody, antibody fragment or derivative thereof may be, e.g. DNA, cDNA, RNA or synthetically produced DNA or RNA or recombinantly produced chimeric nucleic acid molecule comprising any of those nucleic acid molecules either alone or in combination. The nucleic acid molecule may also be genomic DNA corresponding to the entire gene or a substantial portion thereof or to fragments and derivatives thereof. The nucleotide sequence may correspond to the naturally occurring nucleotide sequence or may contain single or multiple nucleotide substitutions, deletions or additions. In a particular preferred embodiment of the present invention, the nucleic acid molecule is a cDNA molecule.

The term "hybridizing under stringent conditions" means that two nucleic acid fragments hybridize with one another under standardized hybridization conditions as described for example in Sambrook et al., "Expression of cloned genes in E. coli" in Molecular Cloning: A laboratory manual (1989), Cold Spring Harbor Laboratory Press, New York, USA. Such conditions are for example hybridization in 6.0xSSC at about 45° C. followed by a washing step with 2.0xSSC at 50° C, preferably 2.0xSSC at 65°C, or 0.2xSSC at

50°C, preferably 0.2xSSC at 65°C.

Another aspect of the invention relates to a vector comprising a nucleic acid molecule of the invention. Said vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. Preferably, the vector of the invention is an expression vector wherein the nucleic acid molecule is operatively linked to one or more control sequences allowing the transcription and optionally expression in prokaryotic and/or eukaryotic host cells.

The invention further relates to a host comprising the vector of the invention. Said host may be a prokaryotic or eukaryotic cell or a non-human transgenic animal. The polynucleotide or vector of the invention which is present in the host may either be integrated into the genome of the host or it may be maintained extrachromosomally.

The host can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal, mammalian or, preferably, human cell. Preferred fungal cells are, for example, those of the genus Saccharomyces, in particular those of the species S. cerevisiae.

The invention additionally relates to a method for the preparation of an antibody, comprising culturing the host of the invention under conditions that allow synthesis of said antibody and recovering said antibody from said culture.

A further aspect of the present invention relates to a pharmaceutical composition comprising the antibody of the invention or a fragment thereof, the nucleic acid molecule, the vector, the host of the invention or an antibody obtained by a method of the invention. The term "composition" as employed herein comprises at least one compound of the invention. Preferably, such a composition is a therapeutical/pharmaceutical or a diagnostic composition.
The diagnostic composition of the invention may be used for assessing the

onset or the disease status of a hyperproliferative disease as defined herein.

The composition preferably comprises an pharmaceutically acceptable carrier, diluent and/or excipient.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers, excipients and/or diluents can be formulated by well known conventional methods.

Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. Preferred is an intravenous, intramuscular and/or subcutaneous administration.

These pharmaceutical compositions can be administered to the subject at a suitable dose. The dosage regimen can be determined by the attending physician and clinical factors.

The compositions of the invention may be administered locally or systemically. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or' fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents depending on the intended use of the pharmaceutical composition.

According to an especially preferred embodiment the composition comprises a further active agent, such as a further antibody or antibody fragment.

Preferably the composition of the invention is used in combination with at least one further antineoplastic agent. Said combination is effective, for example, in inhibiting abnormal cell growth. Many antineoplastic agents are presently known in the art. In general the term includes all agents that are capable of prevention, alleviation and/or treatment of hyperproliferative disorders. Especially preferred are antineoplastic agents inducing apoptosis.

Preferably the antineoplastic agent is selected from the group consisting of antibodies, small molecules, antimetabolites, alkylating agents, topoisomerase inhibitors, microtubule-targeting agents, kinase inhibitors, protein synthesis inhibitors, immuno-therapeutics, hormones or analogs thereof, and/or mTOR inhibitors.

Specific examples of antineoplastic agents which can be used in combination with the antibodies provided herein include, for example, gefitinib, lapatinib, sunitinib, pemetrexed, bevacisumab, cetuximab, imatinib, alemtuzumab, trastuzumab, rituximab, erlotinib, bortezomib and the like, in particular trastuzumab. Other specific antineoplastic agents to be used in the compositions as described and claimed herein include for example, chemotherapeutic agents such as Paclitaxel, Anthracyclines, Fluoropirimidine, vinca alkaloids, platinum salts, in particular capecitabine, daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide, trimetrexate, teniposide, cisplatin and diethylstilbestrol (DES).

The compositions of the invention may be administered in combination with a further therapeutic composition comprising an active agent as described above and/or irradiation and/or radiotherapy.

According to a preferred embodiment, the compositions of the invention are for the use in treating and/or preventing hyperproliferative diseases, in particular neoplastic diseases or cancer. The compositions may also be used for the manufacture of a medicament for treating and/or preventing hyperproliferative diseases, in particular neoplastic diseases or cancer.

A hyperproliferative disease as defined herein includes any neoplasia, i.e. any abnormal and/or uncontrolled new growth of tissue. The term "uncontrolled new growth of tissue" as used herein may depend upon a dysfunction and/or loss of growth regulation. A hyperproliferative disease includes tumor diseases and/or cancer, such as metastatic or invasive cancers.

The hyperproliferative disease is preferably selected from disorders associated with, accompanied by or caused by ErbB3 expression, overexpression or hyperactivity, such as cancer, in particular melanoma, breast cancer, ovarian cancer, renal carcinoma, gastrointestinal/colon cancer, lung cancer, clear cell carcinoma of the kidney and/or prostate cancer. In particular, for these tumors, it has been demonstrated a role of ErbB3 in promoting cancer development and growth, and, thus, the inhibition of this protein could give certain benefits.

The invention further relates to a method of treating a disease wherein the antibody of the invention is administered to a mammal and wherein said disease is correlated directly or indirectly with an abnormal level of expression or activity of ErbB3.

A further aspect of the present invention relates to a method of inhibiting EGF-like ligand mediated phosphorylation of ErbB3 in a subject, comprising administering to the subject an antibody or antigen binding portion thereof of as described above, in an amount sufficient to inhibit EGF-like mediated phosphorylation of ErbB3. "Phosphorylation of ErbB3" refers to the phosphorylation of amino acid residues, preferably tyrosine residues.

Yet another aspect of the present invention is directed to a method of diagnosing a cancer associated with ErbB3 in a subject, comprising

(a) contacting ex vivo or in vivo cells from the subject with an antibody or antigen binding portion thereof of any one of the preceding claims and

(b) measuring the level of binding to ErbB3 on the cells, wherein abnormally high levels of binding to ErbB3 indicate that the subject has a cancer associated with ErbB3.

In terms of the present invention, "abnormally high" means higher binding levels of ErbB3 compared to a healthy subject having no cancer.

Preferably the subject is an animal, more preferably a mammalian and in particular preferably a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that MP-RM-1 reduces the expression of ErbB3 receptor on the surface of breast cancer cells

Figure 2 shows that MP-RM-1 downregulates ErbB3 receptor in breast cancer cells.

Figure 3 shows that MP-RM-1 is able to reduce ErbB3 receptor half-life in MDA-MB-435 human breast cancer cells.

Figure 4 shows that MP-RM-1 is able to reduce ErbB3 receptor half-life in SKBR-3 human breast cancer cells.

Figure 5 shows that the effect MP-RM-1 on the reduction of the ErbB3 receptor half-life is blocked by the lysosome inhibitor chloroquine.

Figure 6 shows that MP-RM-1 inhibits the phosphorylation of ErbB3 and AKT induced by the receptor ligand NRG-1 in MDA-MB-435 human breast cancer and IR-8 human melanoma cells.

Figure 7 shows that ligand-induced phosphorylation of ErbB3 and AKT is inhibited by MP-RM-1 in a time-dependent fashion.

Figure 8 shows that MP-RM-1 is able to antagonize the ligand-induced activation of ErbB3 and AKT.

Figure 9 shows that MP-RM-1 is rapidly (30 minutes) internalized into cells.

Figure 10 compares the effect of MP-RM-1 and Trastuzumab on ligand-induced activation of ErbB3 and AKT in human breast and prostate cancer cells.

Figure 11 shows that MP-RM-1 is able to inhibit basal ErbB3 and AKT phoshphorylation in MET-amplified MKN-45 human gastric cancer cells.

Figure 12 shows that MP-RM-1 inhibits ligand-induced proliferation in MDA-MB-435 human breast cancer cells.

Figure 13 shows that MP-RM-1 inhibits the growth of DU145 human prostate cancer xenografts

Figure 14 shows that as soon as 4 hours after injection into mice, MP-RM-1 induces ErbB3 downregulation and inhibits AKT activation in IR-8 human melanoma xenografts..

Figure 15 shows that treatment of IR-8 human melanoma cells with cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10, hMP-RM-1 #20 MP-RM-1 antibody variants reduces the expression of ErbB3 receptor on cell surface.

Figure 16 shows that the humanized variant hMP-RM-1 #6 is rapidly (30 minutes) internalized into the cells.

Figure 17 shows the effect of the chimeric variant cMP-RM-1 #1, and the humanized variants hMP-RM-1 #6, hMP-RM-1 #10, hMP-RM-1 #20 antibody variants on ErbB3 half-life in IR-8 human melanoma cells.

Figure 18 shows the inhibitory effect of cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 and hMP-RM-1 #20 antibody variants on the phosphorylation of ErbB3 and AKT in human ovarian (A) and gastric (B) cancer cells.

Figure 19 shows the inhibitory effect of cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 and hMP-RM-1 #20 antibody variants on human melanoma (A) or gastric cancer (B) cell colony formation in soft agar.

Figure 20 shows that tumor xenografts of mice treated with cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 and hMP-RM-1 #20 antibody variants grow significantly less than those of control mice.

Examples

Example 1: Production of the monoclonal antibody MP-RM-1.

Four-weeks old Balb/c mice were immunized by intraperitoneal injection of live NIH/3T3 cells transfected with the human ErbB3 receptor. Seven days later, mice were given an additional intraperitoneal injection of the immunogen. After additional seven days, mice were boosted intravenously with the immunogen, and spleens were removed for cell fusion 3 days later. Somatic cell hybrids were prepared by fusion of immune splenocytes with the murine nonsecreting myeloma cell line NS-1. Hybridoma supernatants were selected on the basis of differential reactivity with LTR-ErbB3 transfected cells, but not with LTR-neo NIH/3T3. All positive hybridoma cell colonies were cloned twice by limiting dilution and further characterized. The selected monoclonal antibody, named MP-RM-1 (isotype IgG2a) was found to specifically recognize the extracellular domain of the ErbB3 receptor.

The hybridoma murine cell line producing MP-RM-1 antibody was deposited at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) and designated DSM ACC3018.

Example 2: Effect of MP-RM-1 on ErbB3 receptor expression on the surface of breast cancer cells.

<u>Materials and methods:</u> MDA-MB-435 human breast cancer cells were maintained on ice for 30 minutes with 10 µg/ml of MP-RM-1 and then returned to 37°C. At the indicated times, cells were trypsinized and stained with a fluorescein-labeled goat anti-mouse IgG antibody and analyzed by FACS.

Results: MP-RM-1 decreases ErbB3 receptor expression on the cell surface in a time-dependent manner (Figure 1).

Example 3: Effect of MP-RM-1 on downregulation of the ErbB3 receptor in breast cancer cells.

Materials and methods: MDA-MB-435 human breast cancer cells were grown in 0.2% FBS DMEM for 24 hours and then incubated in the presence or absence of 10 µg/ml of MP-RM-1 for 15, 60, 120 and 240 minutes. At the end of the incubation periods, cells were lysed and analyzed for ErbB3 and AKT protein levels by Western blotting with anti-ErbB3 and anti-AKT. The same filter was reprobed with anti-PLC γ -1 for a loading control.

Results: MP-RM-1 induces downregulation of ErbB3 receptor after 120 minutes (Figure 2).

Example 4: Effect of MP-RM-1 on ErbB3 receptor half-life in breast cancer cells.

Materials and methods: MDA-MB-435 human breast cancer cells were grown in 0.2% FBS DMEM for 24 hours and then chased with the protein synthesis inhibitor cycloheximide at 10 µg/ml with or without MP-RM-1. Cells were lysed and analyzed for ErbB3 and AKT protein levels by Western blotting with anti-ErbB3 and anti-AKT. The same filter was reprobed with anti- PLC γ -1 or anti-Actin for a loading control.

Results: ErbB3 receptor half-life is markedly reduced in cycloheximide chased, MP-RM-1 treated MDA-MB-435 cells compared to PBS-treated control cells (Figure 3).

Example 5: Effect of MP-RM-1 on ErbB3 receptor half-life in breast

cancer cells.

Materials and methods: SKBR-3 human breast cancer cells were grown in 0.2% FBS DMEM for 24 hours and then chased with the protein synthesis inhibitor cycloheximide at 10 µg/ml in the presence or absence of MP-RM-1. Cells were lysed and analyzed for ErbB3 levels by Western blotting with anti-ErbB3.

Results: ErbB3 receptor half-life is markedly reduced in cycloheximide chased, MP-RM-1 treated SKBR-3 cells compared to the PBS treated control cells (Figure 4).

Example 6: Effect of chloroquine on ErbB3 receptor downregulation induced by MP-RM-1.

Materials and Methods: MDA-MB-435 human breast cancer cells were grown on 0.2% FBS for 24 hours and chased for 3 hours with cycloheximide at 10 µg/ml. Cells were then incubated with MP-RM-1 in the presence or absence of chloroquine. After incubation, cells were lysed and analyzed for ErbB3 protein levels by Western blotting with anti-ErbB3. The same filter was reprobed with anti-Actin for a loading control

Results: ErbB3 receptor downregulation induced by MP-RM-1 is blocked by chloroquine (Figure 5).

Example 7: Effect of MP-RM-1 on ligand-induced ErbB3 and AKT phosphorylation in breast and melanoma cancer cells.

Materials and methods: MDA-MB-435 human breast cancer cells, A375 and IR-8 human melanoma cells were grown in 0.2% FBS in DMEM or RPMI for 24 hours. Cells were incubated in the presence or absence of MP-RM-1 at 1 or 10 µg/ml for 2 hours and then stimulated with 10 ng/ml of NRG-1 for 5 minutes. After incubation, cells were lysed and analyzed for ErbB3, p-ErbB3, AKT, p-AKT, or p-Erks protein levels by Western blotting with anti-ErbB3, anti-p-ErbB3, anti-AKT, anti-p-AKT and anti-p-Erks. The same filter was reprobed with anti-Actin for a loading control.

Results: Cells pre-treated with MP-RM-1 exhibit a dose-dependent inhibition of ErbB3 and AKT ligand-induced phosphorylation (Figure 6).

Example 8: Effect of MP-RM-1 on ligand-induced ErbB3 and AKT phosphorylation.

Materials and methods: MDA-MB-435 human breast cancer cells were grown in 0.2% FBS DMEM for 24 hours and then stimulated with 10 ng/ml of NRG-1 for 5 minutes. Cells were then incubated with 10 µg/ml of MP-RM-1 for 15, 60 and 120 minutes before of NRG-1 stimulation. After incubation, cells were lysed and analyzed for ErbB3, p-ErbB3, AKT and p-AKT protein levels by Western blotting with anti-ErbB3, anti-p-ErbB3, anti-AKT and anti-p-AKT.

Results: Cells pre-treated with MP-RM-1 exhibit a time-dependent inhibition of ErbB3 and AKT ligand-induced phosphorylation (Figure 7).

Example 9: Effect of MP-RM-1 on ligand-induced ErbB3 and AKT phosphorylation.

Materials and Methods: MDA-MB-435 human breast cancer cells were grown in 0.2% FBS DMEM for 24 hours and then simultaneously stimulated with 10 ng/ml of NRG-1 and 10 µg/ml of MP-RM-1 for 5 minutes. After incubation, cells were lysed and analyzed for ErbB3, p-ErbB3 and p-AKT protein levels by Western blotting with anti-ErbB3, anti-p-ErbB3 and anti-p-AKT.

Results: 5 minutes stimulation with MP-RM-1 does not induce ErbB3 and AKT phosphorylation, indicating that MP-RM-1 is not a receptor agonist. By contrast, ligand-induced ErbB3 and AKT phosphorylation is partially inhibited by MP-RM-1, indicating that MP-RM-1 is a partial receptor antagonist (Figure 8).

Example 10: MP-RM-1 internalization in MDA-MB-435 breast carcinoma cells.

Materials and Methods: MDA-MB-435 human breast cancer cells cells were plated in 22X22 mm coverslips and grown in 0.2% FBS DMEM for 24 hours. Cells were then incubated with 10 µg/ml of MP-RM-1 for 30 minutes on ice and returned at 37°C. After 30 and 60 minutes, cells were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton-X100 in PBS and then stained with a fluorescein-labeled goat anti-mouse antibody (green staining), phalloydin (red staining). Cell nuclei were counterstained in blue. The yellow and the white arrows indicate MP-RM-1 localization on the cell membrane and in the cytoplasm, respectively.

Results: MDA MB 435 cells show goat anti mouse membrane positivity (yellow arrows) after 30 minutes of MP-RM-1 incubation on ice indicating that MP-RM-1 antibody is completely localized on the plasma membrane. After 30 and 60 minutes 37°C incubation the goat anti mouse signals is totally intracellular (white arrows) indicating that MP-RM-1 has been internalized by the cells (Figure 9).

Example 11: Comparative effect of MP-RM-1 and Trastuzumab on ligand-induced activation of ErbB3 and AKT in breast cancer and prostate cells.

Materials and Methods: MDA-MB-435 human breast cancer cells and DU 145 human prostate cancer cells were grown in 0.2% FBS RPMI for 24 hours and then stimulated with 10 ng/ml of NRG-1 for 5 minutes. Cells were then incubated for 2 hours with either MP-RM-1 at 1 or 10 µg/ml, or Trastuzumab at 10 µg/ml before ligand stimulation. After incubation, cells were lysed and analyzed for ErbB3, p-ErbB3, AKT, p-AKT and p-Erks protein levels by Western blotting with anti-ErbB3, anti-p-ErbB3, anti-AKT, anti-p-AKT and anti-p-Erks.

Results: MP-RM-1 inhibits ligand induced ErbB3 and AKT phosphorylation at the same extent of Trastuzumab. However ErbB3 downregulation is induced by MP-RM-1, but not by Trastuzumab (Figure 10).

Example 12: Effect of MP-RM-1 on MET/ErbB3/AKT signalling axis in MET-amplified gastric cancer cells.

Materials and Methods: MKN-45 human gastric cancer cells were grown in 0.2% FBS DMEM for 24 hours. Cells were then exposed to MP-RM-1 at 1 and 10 µg/ml, or Trastuzumab at 10 µg/ml, or MET inhibitor SU11274 at 0.1, 1 and 10 µg/ml. Cells were then lysed and analyzed for ErbB3, p-ErbB3, AKT, p-AKT and p-MET protein levels by Western blotting with anti-ErbB3, anti-p-ErbB3, anti-AKT, anti-p-AKT and anti-p-MET.
Results: MKN-45 cells show a ligand-independent, MET-dependent phosphorylation of ErbB3 receptor and AKT. MP-RM-1, but not Trastuzumab inhibits this basal activity. Moreover, MP-RM-1 is able to disrupt the ligand-indipendent MET/ErbB3 association in vivo (Figure 11).

Example 13: Effect of MP-RM-1 on ligand-induced proliferation of breast cancer cells.

Materials and Methods: MDA-MB-435 human breast cancer cells were grown in 0.2% FBS RPMI for 24 hours and then incubated with 10 ng/ml of NRG-1 for 48 hours in the presence or absence of MP-RM-1 at 1 or 10 µg/ml. At the end of the incubation, cells were trypsinized and counted.

Results: MP-RM-1 inhibits, in a dose-dependent manner, ligand-induced proliferation of MDA-MB-435 cells (Figure 12).

Example 14: Effect of MP-RM-1 on prostate cancer xenografts

Materials and Methods: human prostate cancer xenografts were established by injecting subcutaneously $5x10^6$ DU145 cells in 5-week old CD1 nude mice. When xenografts were palpable, mice were separated into two groups of 10 animals. The two groups had comparable mean tumor volume. One group received intraperitoneal injection twice per week of 20 mg/kg MP-RM-1 in PBS buffer, whereas the other received PBS only (control group). Tumor volume was monitored every day by a caliper. Error bars indicated SE in each group. * denotes significant difference (P =_0.01)** denotes significant difference (P = 0.006) between MP-RM-1 treated mice and PBS-treated (control) mice.

Results: MP-RM-1 treated mice show up to 60% reduction of tumor volume compared to the control mice (0.42 cm^3 vs 0.96 cm^3) (Figure 13).

Example 15 : In vivo effect of MP-RM-1 on ErbB3 downregulation and AKT phosphorylation in melanoma xenografts.

Materials and Methods: Nude mice harboring IR-8 melanoma xenografts were treated or not (U) with 200 µg of MP-RM-1. After 4 hours, 16 hours, and 24 hours, tumors were collected and homogenized with a Polytron homogenizer in a lysis buffer (w:v, 1:10) containing 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 0.1% NP-40, 250 mM NaCl, 50 mM NaF in the presence of leupeptine, pepstatine, aprotinin and phenyl-methyl-sulfonyl-fluoride. Homogenates were centrifuged at 13,000 rpm for 10 min at 4°C. Aliquots of the supernatants were analyzed for ErbB3, AKT and p-AKT protein levels by Western blotting with anti-ErbB3, anti-AKT, anti-p-AKT. The same filter was reprobed with anti-PLC γ-1 for a loading control.

Results: MP-RM-1 induces ErbB3 downregulation and inhbits AKT phosphorylation in melanoma xenografts starting 4 hours after injection to mice (Figure 14).

Example 16: Production of chimeric and humanized versions of the MP-RM-1 antibody

Methods for humanizing non-human antibodies are well known in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following published procedures (27-29), in particular by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework region (FR) residues are substituted by residues from analogous sites in rodent antibodies.

To produce chimeric and humanized versions of the MP-RM-1 antibody, hybridoma cells producing the MP-RM-1 antibody (deposited at DSMZ, and designed DSM ACC3018) were expanded, total RNA extracted and RT-PCR performed to clone and sequence the variable regions of the antibody using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies).

Based on sequence information of the variable region of MP-RM-1 antibody, 20 different variants of said region have been obtained by gene synthesis using standard procedures.
For antibody chimerization, the murine constant regions were replaced with the human constant regions. Two chimeric versions of the heavy chain (HC) were made in an IgG1 context and two chimeric versions of the heavy chain (HC) in an IgG3 context;

For antibody humanization, Complementarity Determining Regions (CDRs) from the murine were grafted in to a human antibody framework.

Sixteen humanized versions of the heavy chain (HC) were made in an IgG1 and LC-kappa context. Each version is characterized by specific point mutations in the FR.

Sequence information:

CHIMERIC SEQUENCES

SEQUENCE 3: CHIMERIC IgG1 HC SEQUENCE

mnfglrliflvltlkgvqcdvqLVESGGDLVKPGGSLKLSCVVSGFTFSTYGMSWVRQTPDRRLEWVATIS
HGDGYTYYPDSVKGRFTISRDNAKNTLHLQMSSLKSEDTAMYYCARHGDYDDDYYAMDYWGQGTSVTFSsa
stkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvp
ssslgtqtyicnvnhkpsntkvdkrvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpap
iektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdg
sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQUENCE 4A: CHIMERIC IgG2 HC SEQUENCE

mnfglrliflvltlkgvqcdvqLVESGGDLVKPGGSLKLSCVVSGFTFSTYGMSWVRQTPDRRLEWVATIS
HGDGYTYYPDSVKGRFTISRDNAKNTLHLQMSSLKSEDTAMYYCARHGDYDDDYYAMDYWGQGTSVTFSsa
stkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvp
ssnfgtqtytcnvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvv
dvshedpevqfnwyvdgmevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiekt
isktkgqprepqvytlppsreemtknqvsltclvkgfypsdisvewesngqpennykttppmldsdgsffl
yskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQUENCE 4B: CHIMERIC IgG3 HC SEQUENCE

mnfglrliflvltlkgvqcdvqLVESGGDLVKPGGSLKLSCVVSGFTFSTYGMSWVRQTPDRRLEWVATIS
HGDGYTYYPDSVKGRFTISRDNAKNTLHLQMSSLKSEDTAMYYCARHGDYDDDYYAMDYWGQGTSVTFSsa
stkgpsvfplapcsrstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvp
ssslgtqtytcnvnhkpsntkvdkrvelktplgdtthtcprcpepkscdtpppcprcpepkscdtpppcpr
cpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfkwyvdgvevhnaktkpreeqynst
frvvsvltvlhqdwlngkeykckvsnkalpapiektisktkgqprepqvytlppsreemtknqvsltclvk
gfypsdiavewessgqpennynttppmldsdgsfflyskltvdksrwqqgnifscsvmhealhnrftqksl
slspgk

SEQUENCE 5: CHIMERIC LC KAPPA SEQUENCE

mesqtqvlisllfwvsgtcgdIVMTQSPSSLTVIAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPK
LLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNEYTYPLTFGAGTKLE**I**krtvaapsvf
ifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadye
khkvyacevthqglsspvtksfnrgec

SEQUENCE 6: CHIMERIC LC LAMBDA SEQUENCE

mesqtqvlisllfwvsgtcgdIVMTQSPSSLTVIAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPK
LLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNEYTYPLTFGAGTKL**TV**Lgqpkaapsv
tlfppsseelqankatlvclisdfypgavtvawkadsspvkagvetttpskqsnnkyaassylsltpeqwk

shrsyscqvthegstvektvaptecs

HUMANIZED SEQUENCES

SEQUENCE 7: HUMANIZED IgG1 HC SEQUENCE 1

mnfglrliflvltlkgvqcdvqLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQTPDKRLEWVATIS
HGDGYTYYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHGDYDDDYYAMDYWGQGTLVTVSsa
stkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvp
ssslgtqtyicnvnhkpsntkvdkrvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpap
iektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdg
sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQUENCE 8: HUMANIZED IgG1 HC SEQUENCE 2

mnfglrliflvltlkgvqcdvqLVESGGGLVQPGGSLRLSCAVSGFTFSTYGMSWVRQAPGKGLEWVATIS
HGDGYTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHGDYDDDYYAMDYWGQGTLVTVSsa
stkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvp
ssslgtqtyicnvnhkpsntkvdkrvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpap
iektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdg
sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQUENCE 9: HUMANIZED IgG1 HC SEQUENCE 3

mnfglrliflvltlkgvqcdvqLVESGGDLVKPGGSLKLSCVASGFTFSTYGMSWVRQTPDKRLEWVATIS
HGDGYTYYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHGDYDDDYYAMDYWGQGTTVTVSsa
stkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvp
ssslgtqtyicnvnhkpsntkvdkrvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpap
iektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdg
sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQUENCE 10: HUMANIZED IgG1 HC SEQUENCE 4

mnfglrliflvltlkgvqcdvqLVESGGDLVKPGGSLKLSCVASGFTFSTYGMSWVRQTPDRRLEWVATIS
HGDGYTYYPDSVKGRFTISRDNAKNTLHLQMSSLKSEDTAMYYCARHGDYDDDYYAMDYWGQGTTVTVSsa
stkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvp
ssslgtqtyicnvnhkpsntkvdkrvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpap
iektiskakgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdg
sfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQUENCE 11: HUMANIZED LC KAPPA SEQUENCE 1

mesqtqvlisllfwvsgtcgdIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPK
LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNEYTYPLTFGGGTKLEIkrtvaapsvf
ifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadye
khkvyacevthqglsspvtksfnrgec

SEQUENCE 12: HUMANIZED LC KAPPA SEQUENCE 2

mesqtqvlisllfwvsgtcgdIQMTQSPSSLSASVGDRVTITCKSSQSLLNSGNQKNYLTWYQQKPGKAPK

LLIYWASTRESGVP**S**RF**S**GSGSGTDFTLTISS**LQP**EDF**AT**YYCQNEYTYPLTFG**Q**GTK**VEI***krtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec*

SEQUENCE 13: HUMANIZED LC KAPPA SEQUENCE 3

mesqtqvlisllfwvsgtcgdIVMTQSP**D**SLTV**SL**GE**RA**T**IN**CKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFSG**S**GSGTDFTLTISSVQAED**V**AVYYCQNEYTYPLTFG**G**GTKLEL*krtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec*

SEQUENCE 14: HUMANIZED LC KAPPA SEQUENCE 4

mesqtqvlisllfwvsgtcgdIVMTQSPSSLTV**SL**GE**RA**TMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFSG**S**GSGTDFTLTISSVQAED**V**AVYYCQNEYTYPLTFG**G**GTKLEL*krtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec*

- Signal peptide in lower case (non italic)
- Variable regions in capital letters, CRDs underlined
- Constant regions in lower case italics
- Point mutations in bold

The 4 chimeric and the 16 humanized synthetic genes were placed into the pCDNA3.1 plasmid expression vector, and then transfected into Chinese Hamster Ovary-S (CHO-S) cells to obtain the synthesis of the monoclonal antibodies. Table 1 shows the 20 different vector combinations and the relative antibodies names.

Table 1. Vector combination of the 20 different variants of the MP-RM-1 antibody

Vector combination	Antibody name
pCDNA3.1 HC-IgG1 CHIM + pCDNA3.1 LC-kappa CHIM	cMP-RM-1 #1
pCDNA3.1 HC-IgG1 CHIM + pCDNA3.1 LC-lamda CHIM	cMP-RM-1 #2
pCDNA3.1 HC-IgG3 CHIM + pCDNA3.1 LC-kappa CHIM	cMP-RM-1 #3
pCDNA3.1 HC-IgG3 CHIM + pCDNA3.1 LC-lamda CHIM	cMP-RM-1 #4
pCDNA3.1 HC-IgG1 HU1 + pCDNA3.1 LC-kappa HU1	hMP-RM-1 #5
pCDNA3.1 HC-IgG1 HU1 + pCDNA3.1 LC-kappa HU2	hMP-RM-1 #6
pCDNA3.1 HC-IgG1 HU1 + pCDNA3.1 LC-kappa HU3	hMP-RM-1 #7
pCDNA3.1 HC-IgG1 HU1 + pCDNA3.1 LC-kappa HU4	hMP-RM-1 #8
pCDNA3.1 HC-IgG1 HU2 + pCDNA3.1 LC-kappa HU1	hMP-RM-1 #9
pCDNA3.1 HC-IgG1 HU2 + pCDNA3.1 LC-kappa HU2	hMP-RM-1 #10
pCDNA3.1 HC-IgG1 HU2 + pCDNA3.1 LC-kappa HU3	hMP-RM-1 #11
pCDNA3.1 HC-IgG1 HU2 + pCDNA3.1 LC-kappa HU4	hMP-RM-1 #12
pCDNA3.1 HC-IgG1 HU3 + pCDNA3.1 LC-kappa HU1	hMP-RM-1 #13
pCDNA3.1 HC-IgG1 HU3 + pCDNA3.1 LC-kappa HU2	hMP-RM-1 #14
pCDNA3.1 HC-IgG1 HU3 + pCDNA3.1 LC-kappa HU3	hMP-RM-1 #15
pCDNA3.1 HC-IgG1 HU3 + pCDNA3.1 LC-kappa HU4	hMP-RM-1 #16
pCDNA3.1 HC-IgG1 HU4 + pCDNA3.1 LC-kappa HU1	hMP-RM-1 #17
pCDNA3.1 HC-IgG1 HU4 + pCDNA3.1 LC-kappa HU2	hMP-RM-1 #18

pCDNA3.1 HC-IgG1 HU4 + pCDNA3.1 LC-kappa HU3	hMP-RM-1 #19
pCDNA3.1 HC-IgG1 HU4 + pCDNA3.1 LC-kappa HU4	hMP-RM-1 #20

c, chimeric antibody; h, humanized antibody

Initial Screening for Antibodies with the Desired Properties

The supernatants containing the 20 different antibody variants were tested for their ability to inhibit ligand-induced ErbB3 and Akt phosphorylation and to promote ErbB3 down-regulation in IR-8 human melanoma cells. The inhibitory effect of the variants on phosphorylation were evaluated in a long-term assay (treatment of the cells with the antibody variants at 10 μg/ml for 2 hours before NRG-1 stimulation) or in a short-term assay (co-exposure to the antibody variants and NRG-1 for 5 min). The results indicate that 4 antibody variants, i.e., cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10, hMP-RM-1 #20 were the most active in inhibiting ErbB3 and AKT phosphorylation and ErbB3 downregulation both in a long-term and a short-term assay (Table 2).

Table 2. Screening of the MP-RM-1 antibody variants

AMP-RM-1 antibody variants	Isotype	Inhibitory Effect (LT)[1]	Inhibitory Effect (ST)[2]	Downregulation[3]
cMP-RM-1 #1	IgG1	+++	++++	+++
cMP-RM-1 #2	IgG1	+++	+	++
cMP-RM-1 #3	IgG3	+++	+	++
cMP-RM-1 #4	IgG3	++++	+/-	+
hMP-RM-1 #5	IgG1	+++	++	+
hMP-RM-1 #6	IgG1	+++	++++	+++
hMP-RM-1 #7	IgG1	+++	++++	++
hMP-RM-1 #8	IgG1	+++	++++	++
hMP-RM-1 #9	IgG1	+++	++++	++
hMP-RM-1 #10	IgG1	+++	++++	+++
hMP-RM-1 #11	IgG1	+++	++++	++
hMP-RM-1 #12	IgG1	+++	++++	++
hMP-RM-1 #13	IgG1	+++	++	++
hMP-RM-1 #14	IgG1	++++	++	++
hMP-RM-1 #15	IgG1	+++	++	++
hMP-RM-1 #16	IgG1	+++	+	++
hMP-RM-1 #17	IgG1	++++	++	++
hMP-RM-1 #18	IgG1	+++	++	+
hMP-RM-1 #19	IgG1	+++	++++	++
hMP-RM-1 #20	IgG1	++++	++++	++

[1]Refers to the phosphorylation status of ErbB3 and Akt of IR-8 cells that were incubated for 2 hours with the antibody variant after stimulation for 5 min with NRG-1; [2]Refers to the phosphorylation status of ErbB3 and AKT of IR-8 cells that were incubated simultaneously with the antibody variant and NRG-1 for 5 min; [3]Refers to the ability of the antibody variant to promote ErbB3 downregulation as evaluated by western blotting and FACS analysis.

On the basis of these results, the 4 antibody variants cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10, and hMP-RM-1 #20 were selected, purified by means of Protein A capture (HiTrap Protein A HP, GE Healthcare), and further tested for their ability to promote ErbB3 receptor internalization, ErbB3 receptor down-regulation as well as to inhibit in vitro and in vivo growth of human tumor cells.

Example 17: Effect of chimeric and humanized MP-RM-1 antibody variants on ErbB3 receptor expression on the surface of human melanoma cells.

Materials and methods: IR-8 human melanoma cells were maintained on ice for 30 minutes in the presence of 10 µg/ml of chimeric (cMP-RM-1 #1) or humanized (hMP-RM-1 #6, hMP-RM-1 #10, hMP-RM-1 #20) MP-RM-1 antibody variants and then returned to 37°C for 60 minutes. Cells were harvested and stained with a fluorescein-labeled goat anti-human IgG antibody and analyzed by FACS.

Results: chimeric and humanized MP-RM-1 antibody variants induce a decrease of ErbB3 receptor expression on the surface of IR-8 human melanoma cells (Figure 15).

Example 18: Internalization of hMP-RM-1 #6 in human melanoma cells.

Materials and Methods: IR-8 human melanoma cells were plated in 15X15 mm coverslips and grown in 0.2% FBS in RPMI for 24 hours. Cells were then incubated with 10 µg/ml of the humanized MP-RM-1 #6 for 30 minutes on ice and returned at 37°C. After 30 minutes, cells were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton-X100 in PBS and then stained with a fluorescein-labeled goat anti-human antibody (green), phalloidin (red). Cell nuclei were counterstained in blue. The green staining

indicate humanized MP-RM-1#6 antibody localization.
Results: In IR-8 human melanoma cells maintained in ice, hMP-RM-1 #6 antibody variant localizes on cell membrane (green ring). After shifting cells at 37°C for 30 minutes, the antibody is totally localized intracellularly, indicating internalization (Figure 16). Similar results have been obtained with cMP-RM-1 #1, hMP-RM-1 #10, and hMP-RM-1 #20 (not shown).

Example 19: Effect of chimeric and humanized MP-RM-1 antibody variants on ErbB3 receptor half-life in human melanoma cells.

Materials and methods: IR-8 human melanoma cells were grown in 0.2% FBS in RPMI for 24 hours and then chased with the protein synthesis inhibitor cycloheximide (CHX) at 10 µg/ml in the presence or absence of cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 or hMP-RM-1 #20 antibody variants. Cells were lysed and analyzed for p-ErbB3 protein levels by Western blotting with an anti-p-ErbB3 specific antibody. The same filter was re-probed with anti-AKT for a loading control.
Results: In cycloheximide chased IR-8 human melanoma cells, exposure to the antibody variants markedly reduces ErbB3 receptor half-life as compared to PBS-exposed control cells (Figure 17).

Example 20: Effect of chimeric and humanized MP-RM-1 antibody variants on ErbB3 and AKT phosphorylation and ErbB3 receptor down-regulation in human ovarian and gastric cancer cells.

Materials and Methods: (A) OVCAR-8 human ovarian cancer cells were grown in 0.2% FBS in RPMI for 24 hours. Cells were then pre-treated for 2 hours with cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 or hMP-RM-1 #20 antibody variants at 10 µg/ml and stimulated for 10 minutes with ng/ml of NRG-1 10. Cells were lysed and analyzed for ErbB3, p-ErbB3, AKT, p-AKT levels by Western blotting using anti-ErbB3, anti-p-ErbB3, anti-AKT, anti-p-AKT specific antibodies. (B) MKN-45 human gastric cancer cells were grown in 0.2% FBS in DMEM for 24 hours. Cells were then exposed for 2 hours to cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 or hMP-RM-1 #20 antibody variants at 10 µg/ml, or MET inhibitor SU11274 at 1 µg/ml. Cells were lysed and analyzed for p-MET, p-ErbB3 and ErbB3 levels by Western blotting with anti-p-MET, anti-p-ErbB3 and anti-ErbB3 specific antibodies. The same filter was re-probed with anti-AKT as a loading control.

Results: treatment of OVCAR-8 human ovarian cancer with the antibody variants inhibits ErbB-3 and AKT ligand-induced phosphorylation and promotes down-regulation of ErbB3 receptor (Figure 18). MKN-45 cells show a ligand-independent, MET-dependent phosphorylation of ErbB3 receptor. The MP-RM-1 antibody variants are able to inhibit this basal activity (Figure 18).

Example 21: Chimeric and humanized MP-RM-1 antibody variants inhibit colony formation ability of human melanoma and gastric cancer cells.

Materials and Methods: $1.5x10^4$ IR-8 human melanoma or MKN-45 human gastric cancer cells were suspended in 0.3% agarose in RPMI 1640 containing 10% FBS and layered onto a 2 ml bed of 0.5% agarose in six-well plate dishes. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2. After agarose solidification, 5-10-20 µg/ml of cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 or hMP-RM-1 #20 antibody variants or PBS were added to dishes. Treatment administration is repeated every alternate day. The number of colonies in 4 different microscope field at 10 magnification was determined by light microscopy after 10-16 days.

Results: the indicated MP-RM-1 antibody variants reduce the number and dimensions of IR-8 and MKN-45 colonies in soft agar (Figure 19A-B). The bar chart represents the number of the colonies in each.

Example 22: Effect of chimeric and humanized MP-RM-1 antibody variants on human ovarian cancer xenografts.

Materials and Methods: OVCAR-8 human ovarian cancer xenografts were established by injecting subcutaneously $5x10^6$ cells in 7-week old CD1 nude mice. Two days later, mice were randomized into five groups of 10 animals and injected intraperitoneally twice a week for 4 weeks with vehicle (PBS) or 20 mg/kg of cMP-RM-1 #1, hMP-RM-1 #6, hMP-RM-1 #10 or hMP-RM-1 #20 antibody variants. Tumor volume was monitored by a caliper. Error bars indicated SE in each group. The arrows indicate the start (S) and the end (E) of treatment.

Results: Tumor xenografts of mice with chimeric or humanized antibody variants grow significantly slower than those of control mice ($P < 0.05$). (Figure 20).

References:

1. World Health Statistics, World Health Organization, 2008.
2. A Jemal, R Siegel, E Ward et al. Cancer Statistics, 2009. CA Cancer J Clin 59: 225-49 (2009).
3. Mehlen P, Puisieux A. Metastasis: a question of life or death. Nat Rev Cancer; 6:449-58 (2006).
4. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2:563-72 (2002).
5. Gossage L, Eisen T. Targeting multiple kinase pathways: a change in paradigm. Clin Cancer Res. Mar 9 (2010).
6. N.E. Hynes , MacDonald G. ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol 21(2):177-84 (2009).
7. T.Holbro, G.Civenni, N.E. Hynes, The ErbB receptors and their role in cancer progression. Exp Cell Res 284, 99-110 (2003).
8. Browne BC, O'Brien N, Duffy et al. HER-2 signaling and inhibition in breast cancer. Curr Cancer Drug Targets 9(3): 419-38(2009).
9. Harari D, Yarden Y. Molecular mechanisms underlying ErbB2/ERBB2 action in breast cancer. Oncogene 19 (53) : 6102-14 (2000).
10. Alimandi M, Wang LM, Bottaro D et al. Epidermal growth factor and betacellulin mediate signal transduction through co-expressed ErbB2 and ErbB3 receptors. EMBO J 16(18): 5608-17 (1997).
11. Alimandi M, Romano A, Curia MC, Muraro R et al. Cooperative siganling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas. Oncogene 10(9) : 1813-21 (1995).
12. N.E. Hynes, Targeting ERBB receptors in cancer. Recent Results Cancer Res. 172, 45-57 (2007).
13. N.E. Hynes, H.A. Lane, ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5, 341-54 (2005).
14. J.Baselga and S.M. Swain, Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. Nat.Rev.Cancer. 9,463-75, (2009).
15. B.Tanner, D Hasenclever, K Stern et al. ErbB3 predicts survival in Ovarian cancer. J.Clin.Onco. 24, 17-23 (2006).

16. M.Reschke, D.Mihic-Probst, E.H. van der Horst et al. ERBB3 is a determinant for poor prognosis in Melanoma. Clin.Canc.Res. 14, 5188-97 (2008).

17. M. Soler, F Manicni, O.Meca-Cortes et al. ERBB3 is requie for the maintenance of neuregulin-depndent and -independent attributes of malignant progression in prostate cancer cells. Int.J.Cancer 125,2565-75 (2009).

18. D.F. Stern, ERBB3/ERBB3 and ERBB2/ERBB2 duet in mammamry development and breast cancer. J Mammary Gland Biol Neoplasia. 13, 215-23 (2008).

19. J.A. Engelman, K. Zejnullahu, T Mitsudomi et al. MET amplification leads to Gefitinib resistance in lung cancer by activating ERBB3 signalling. Science 316, 1039-43 (2007).

20. L.M. Weiner, M.V. Dhodapkar and S. Ferrone. Monoclonal antibodies for cancer immunotherapy.

21. X. Huang, L. Gao, S. Wang et al. Heterotrimerization of the growth factor receptors ErbB2. ErbB3, and Insulin-like growth factor-I receptor in breast cancer cells resistant to Herceptin. Canc.Res.

22. T.Holbro, R. Beerli, F. Maurer et al. The ErbB2/ErbB3 heterodimer functions a san oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation.

23. Y Yarden. The EGFR family and its ligands in human cancer: signaling mechanisms and therapeutic opportunities.

24. M.R. Freeman. HER-2/ERBB3 heterodimers in prostate cancer: whither HER1/EGFR ? Cancer Cell 6:427-428 (2004).

25. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495-497.

26. Clackson T, Hoogenboom HR, Griffiths AD, Winter G. Making antibody fragments using phage display libraries. Nature 1991; 15;352(6336):624-8.

27. Jones et al., Replacing the complementarity-determining regions in a human antibody with those from a mouse, Nature, 321:522-525 (1986).

28. Riechmann et al., Reshaping human antibodies for therapy, Nature,

332:323-327 (1988).
29. Verhoeyen et al., Reshaping human antibodies: grafting an antilysozome activity, Science, 239:1534-1536 (1988).

Claim

1. Antibody or fragment thereof which binds to the ErbB3 receptor and which comprises:
   a) a heavy chain amino acid sequence as encoded by SEQ ID NO: **1** or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 95%, thereto, whereby the CDRs are comprising the following amino acid sequences: CDR1 comprises amino acids TYGMS, CDR2 comprises amino acids TISHGDGYTYYPDSVKG, and CDR3 comprises amino acids HGDYDDDYYAMDY and
   b) a light chain amino acid sequence as encoded by SEQ ID NO: **2** or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 95% thereto, whereby the CDRs are comprising the following amino acid sequences: CDR1 comprises amino acids SQSLLNSGNQKNYLT, CDR2 comprises amino acids WASTRES, and CDR3 comprises amino acids QNEYTYPLT.

2. The antibody of claim 1, which is a polyclonal antibody or a monoclonal antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or a fragment thereof.

3. The antibody of claim 2, wherein the monoclonal antibody is a recombinant antibody.

4. The antibody of claim 2, wherein the multispecific antibody is a bispecific antibody.

5. The antibody according to any one of claims 1-4 which is a Fab fragment, a Fab' fragment, a F(ab') fragment, a Fv- fragment, a diabody, ScFv, or SMIP.

6. The antibody according to any one of claims 1-5, which is MP-RM-1, deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and designated DSM ACC3018.

7. The antibody according to any one of claims 1-6, produced from the hybridoma cell line DSM ACC3018.

8. The antibody according to any one of claims 1-5, comprising at least one sequence as shown in SEQ ID NOs: 3-15.

9. The antibody according to any one of claims 1-5, comprising at least one sequence as shown in sequences 7-14.

10. The antibody according to any one of claims 1-5 and 8 which has a sequence combination selected from the group SEQ ID NO: 3 and SEQ ID NO: 6; SEQ ID NO: 3 and SEQ ID NO: 7; SEQ ID NO: 5 and SEQ ID NO: 6; SEQ ID NO: 5 and SEQ ID NO: 7; SEQ ID NO: 8 and SEQ ID NO: 12; SEQ ID NO: 8 and SEQ ID NO: 13; SEQ ID NO: 8 and SEQ ID NO: 14; SEQ ID NO: 8 and SEQ ID NO: 15; SEQ ID NO: 9 and SEQ ID NO: 12; SEQ ID NO: 9 and SEQ ID NO: 13; SEQ ID NO: 9 and SEQ ID NO: 14; SEQ ID NO: 9 and SEQ ID NO: 15; SEQ ID NO: 10 and SEQ ID NO: 12; SEQ ID NO: 10 and SEQ ID NO: 13; SEQ ID NO: 10 and SEQ ID NO: 14; SEQ ID NO: 10 and SEQ ID NO: 15; SEQ ID NO: 11 and SEQ ID NO: 12; SEQ ID NO: 11 and SEQ ID NO: 13; SEQ ID NO: 11 and SEQ ID NO: 14; and SEQ ID NO: 11 and SEQ ID NO: 15.

11. The antibody according to any one of claims 1-10, which is coupled to a labelling group and/or an effector group.

12. A nucleic acid encoding the antibody or antibody fragment as defined in any one of claims 1-10.

13. A vector comprising the nucleic acid of claim 12.

14. A host cell comprising the nucleic acid of claim 12 or the vector of claim 13.

15. A pharmaceutical composition comprising the antibody as defined in any one of claims 1-11, the nucleic acid of claim 12 and/or the vector of claim 13, and a pharmaceutically acceptable carrier, diluent and/or excipient.

16. The antibody or fragment thereof of any one of claims 1-11 for inhibiting EGF-like ligand mediated phosphorylation of ErbB3 in a subject suffering from a cancer-related disorder, wherein the cancer-related disorder is melanoma, breast cancer, ovarian cancer, renal carcinoma, gastro intestinal/colon cancer, lung cancer, clear cell carcinoma of the kidney and/or prostate cancer.

17. The antibody or fragment thereof of any one of claims 1-11 for diagnosing a cancer associated with ErbB3 in a subject.

18. A process of manufacturing the antibody as defined in any one of claims 1-11 comprising the step of obtaining the antibody from the host of claim 14.

19. Use of the antibody as defined in any one of claims 1-11, for diagnosing a cancer associated with ErbB3 in a subject.

20. Use of the antibody as defined in any one of claims 1-11, for inhibiting EGF-like ligand mediated phosphorylation of ErbB3 in a subject suffering from a cancer-related disorder, wherein the cancer-related disorder is melanoma, breast cancer, ovarian cancer, renal carcinoma, gastro intestinal/colon cancer, lung cancer, clear cell carcinoma of the kidney and/or prostate cancer.

21. Use of the pharmaceutical composition as defined in claim 15, for diagnosing a cancer associated with ErbB3 in a subject.

22. Use of the pharmaceutical composition as defined in claim 15, for inhibiting EGF-like ligand mediated phosphorylation of ErbB3 in a subject suffering from a cancer-related disorder, wherein the cancer-related disorder is melanoma, breast cancer, ovarian cancer, renal carcinoma, gastro intestinal/colon cancer, lung cancer, clear cell carcinoma of the kidney and/or prostate cancer.

Figure 1

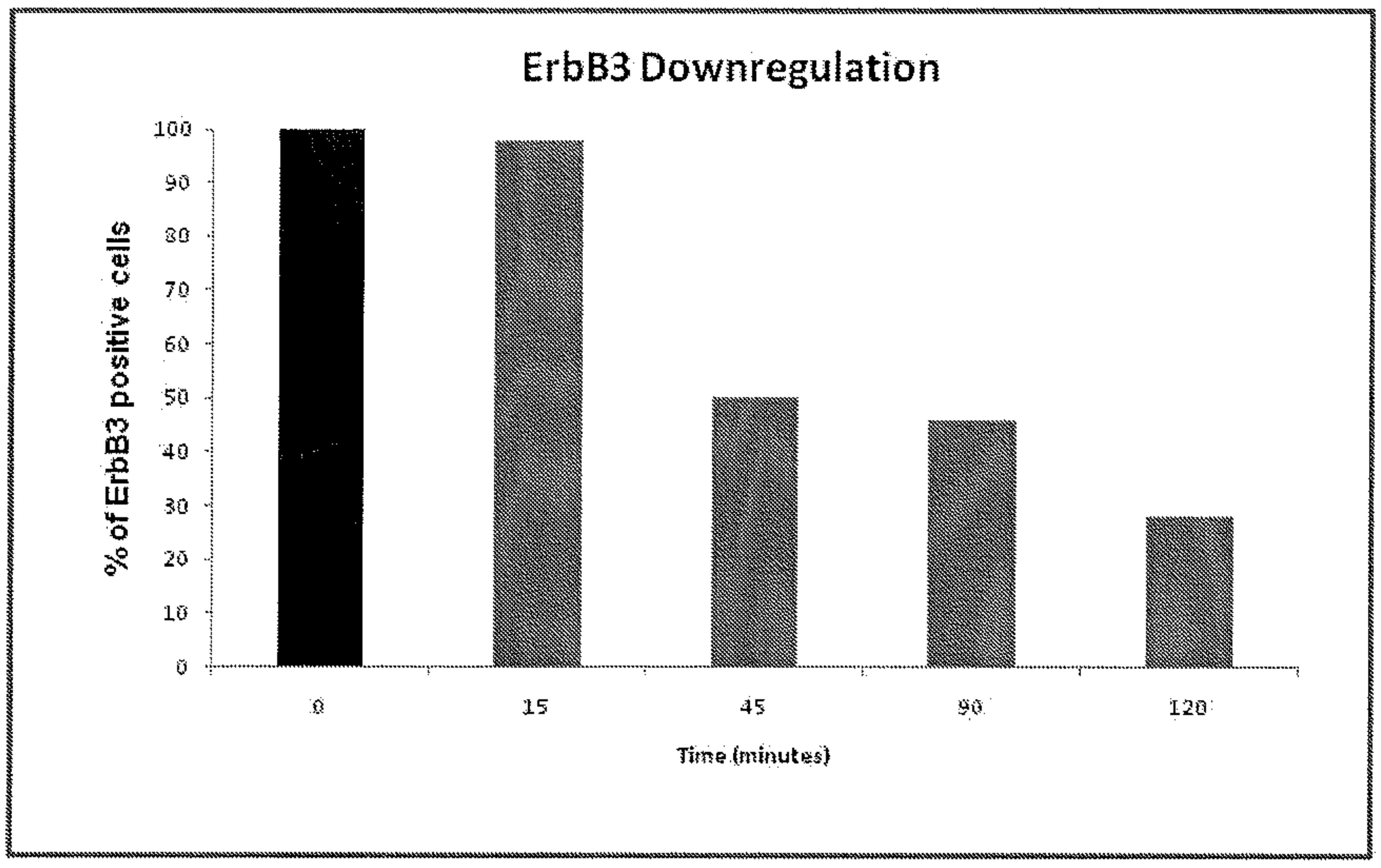

ErbB3 Downregulation
% of ErbB3 positive cells
100
90
80
70
60
50
40
30
20
10
0
0
15
45
90
120
Time (minutes)

Figure 2

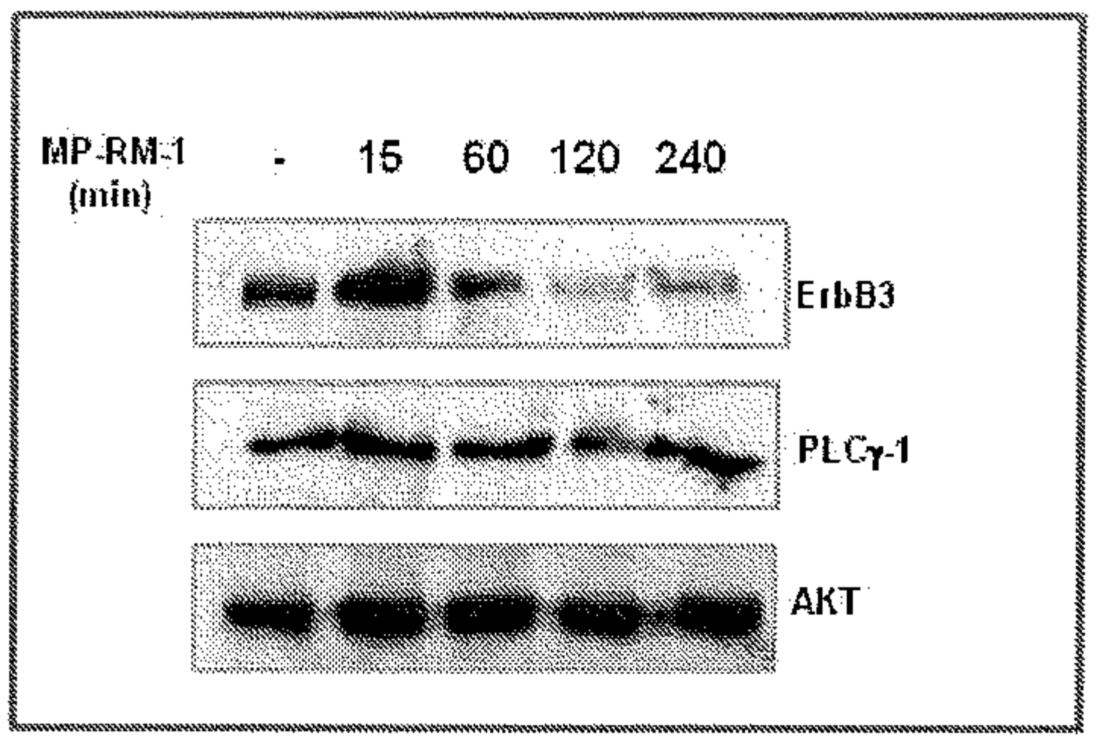


Figure 3

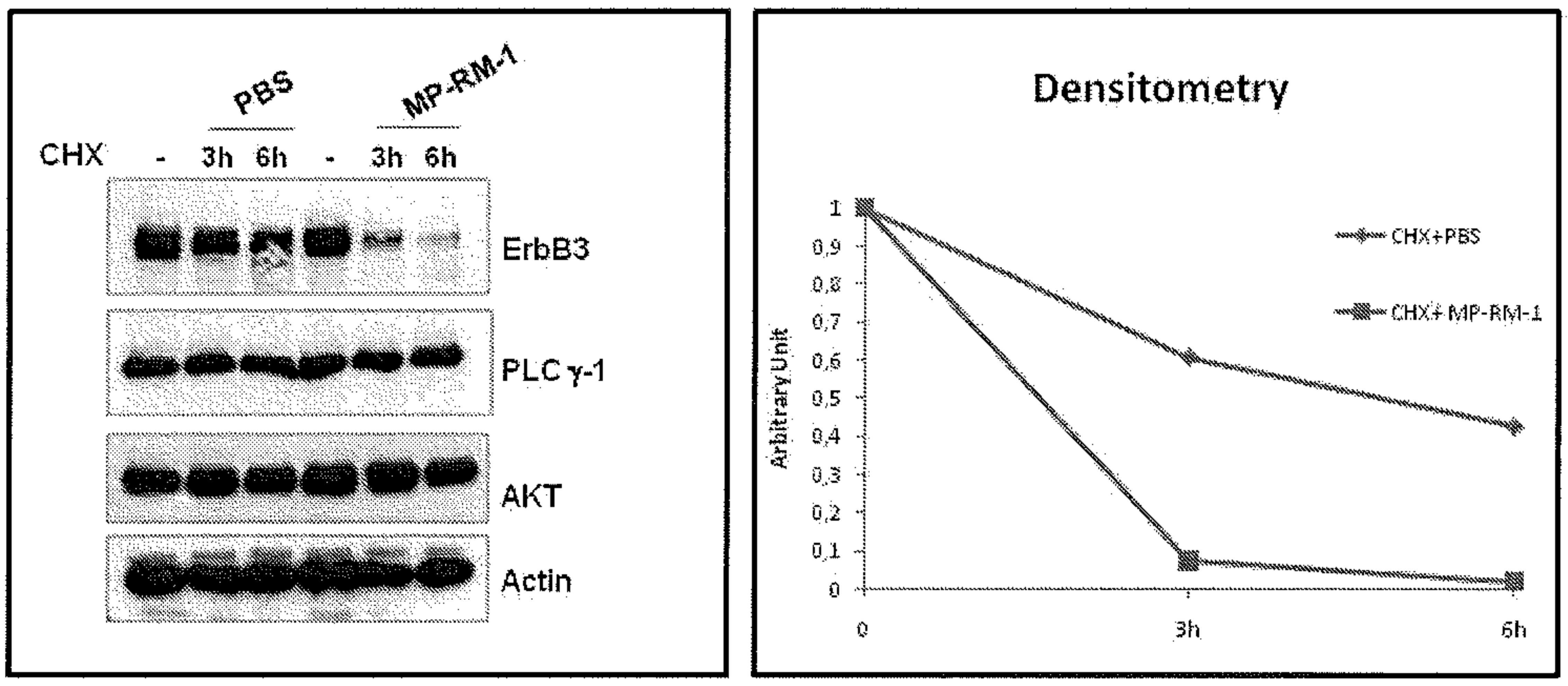

PBS
MP-RM-1
CHX
-
3h
6h
-
3h
6h
ErbB3
PLC γ-1
AKT
Actin
Densitometry
Arbitrary Unit
CHX+PBS
CHX+ MP-RM-1
0
3h
6h

Figure 4

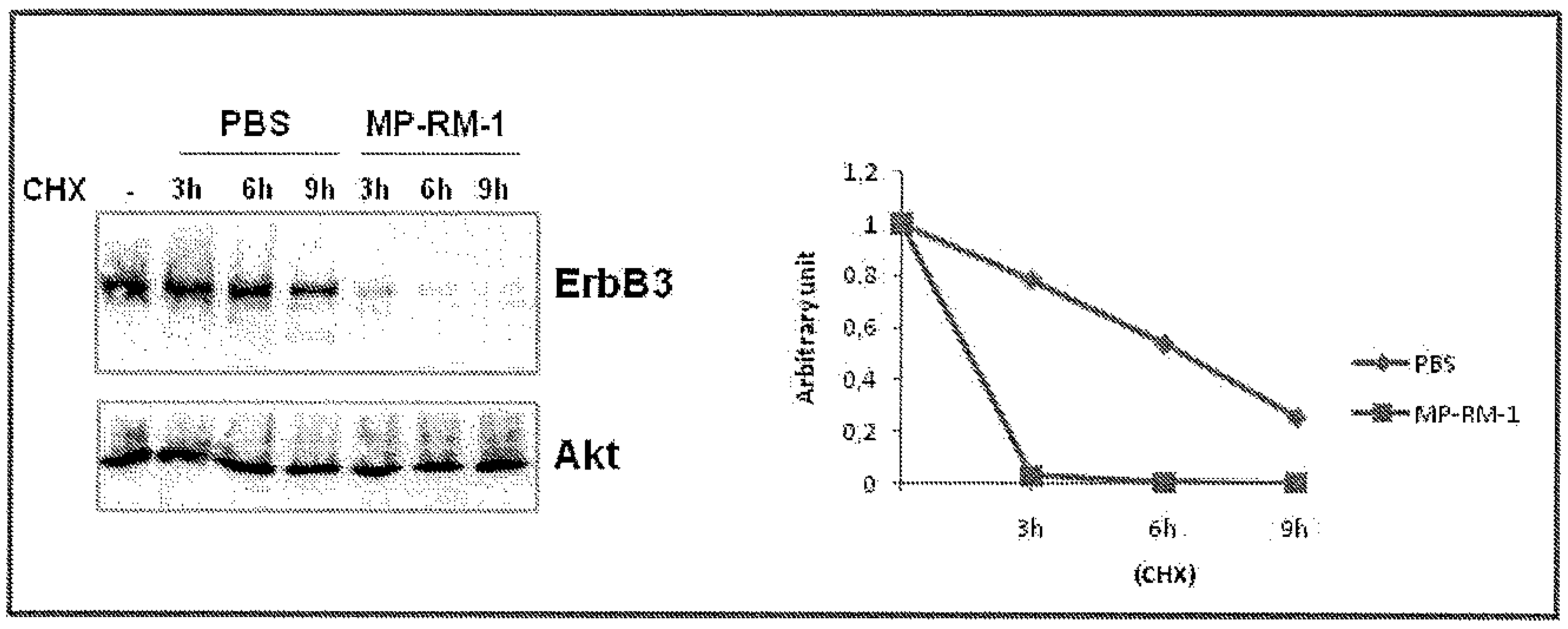

PBS
MP-RM-1
CHX - 3h 6h 9h 3h 6h 9h
ErbB3
Akt
1,2
1
0,8
0,6
0,4
0,2
0
Arbitrary unit
3h
6h
9h
(CHX)
PBS
MP-RM-1

Figure 5

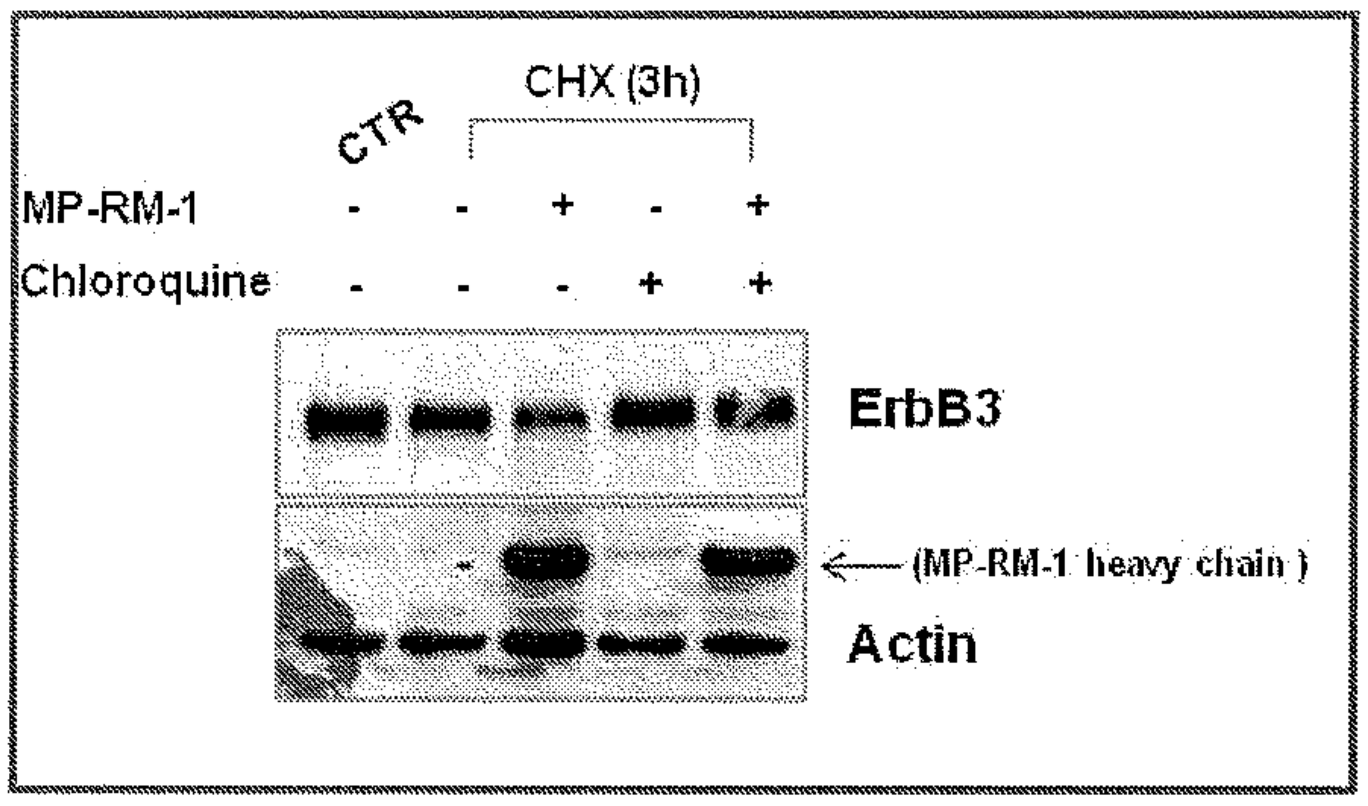


Figure 6

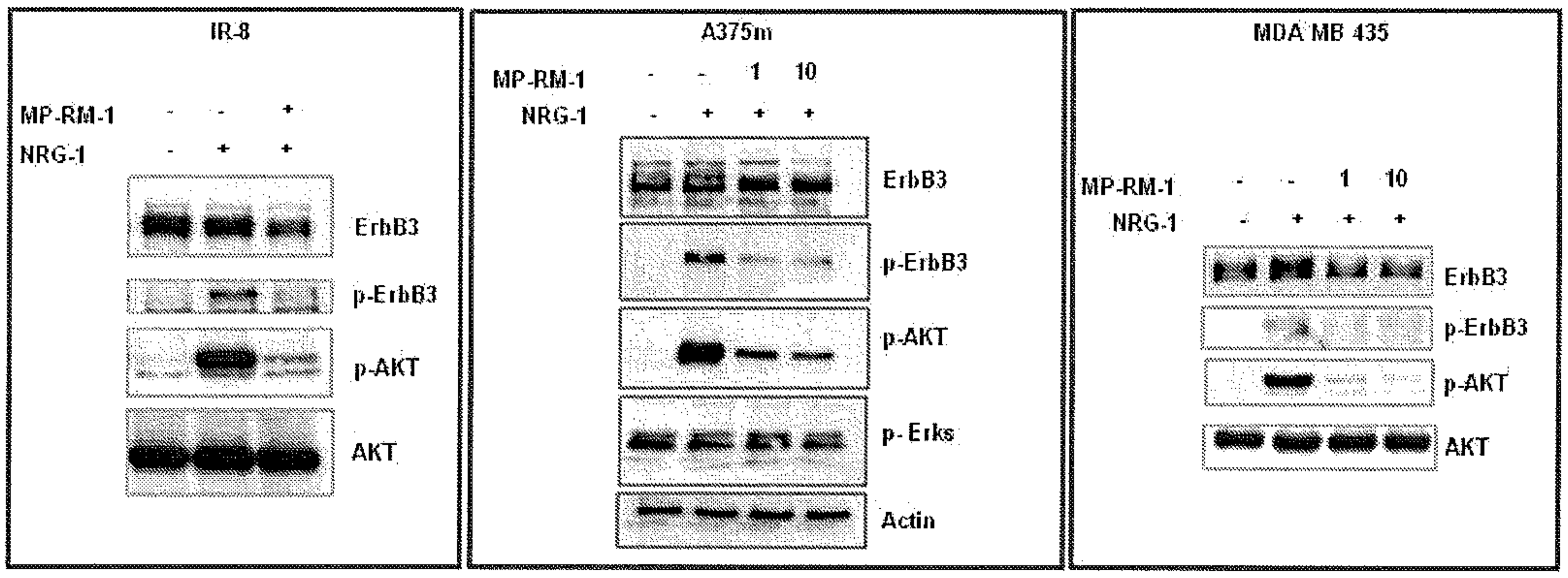

IR-8
MP-RM-1
NRG-1
ErbB3
p-ErbB3
p-AKT
AKT
A375m
MP-RM-1
NRG-1
1
10
ErbB3
p-ErbB3
p-AKT
p- Erks
Actin
MDA MB 435
MP-RM-1
NRG-1
1
10
ErbB3
p-ErbB3
p-AKT
AKT

Figure 7

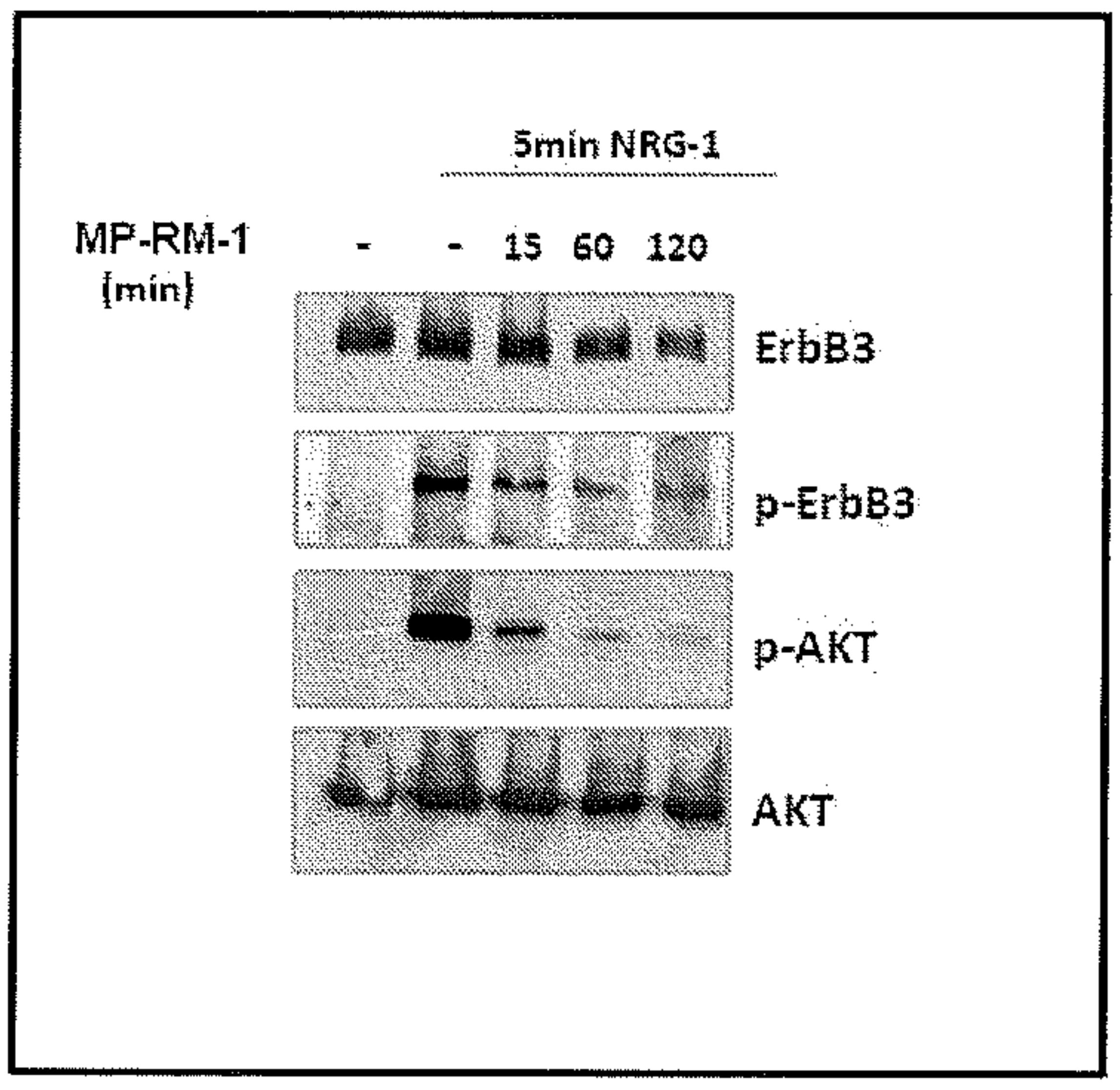


Figure 8

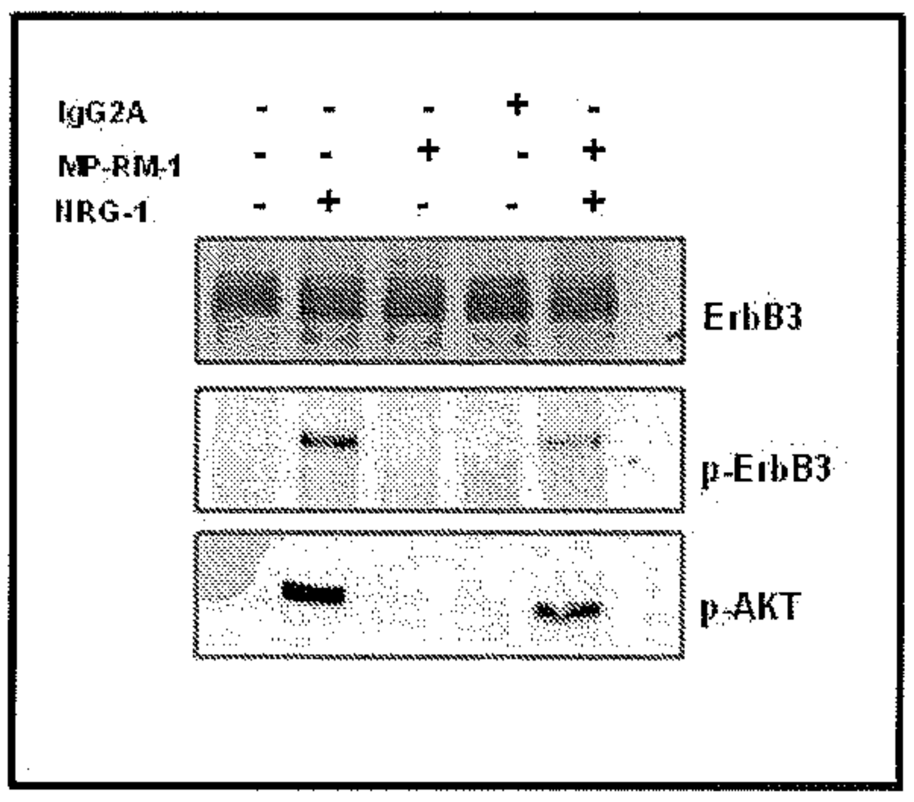


Figure 9

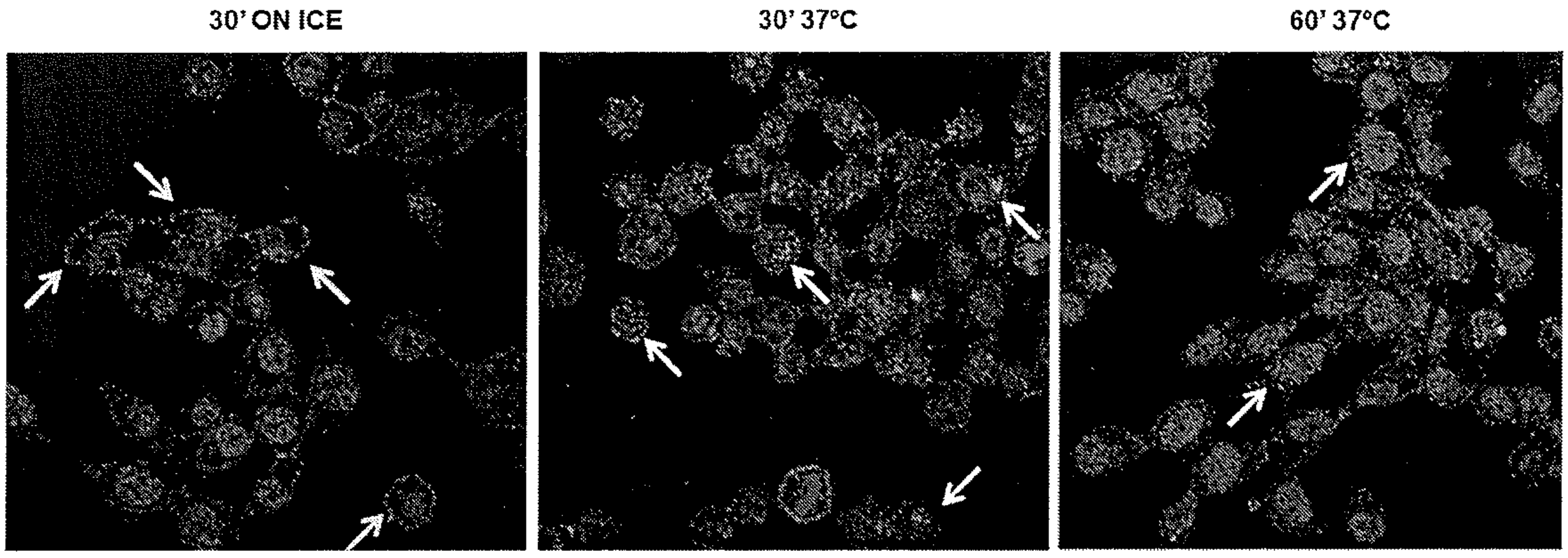


Figure 10

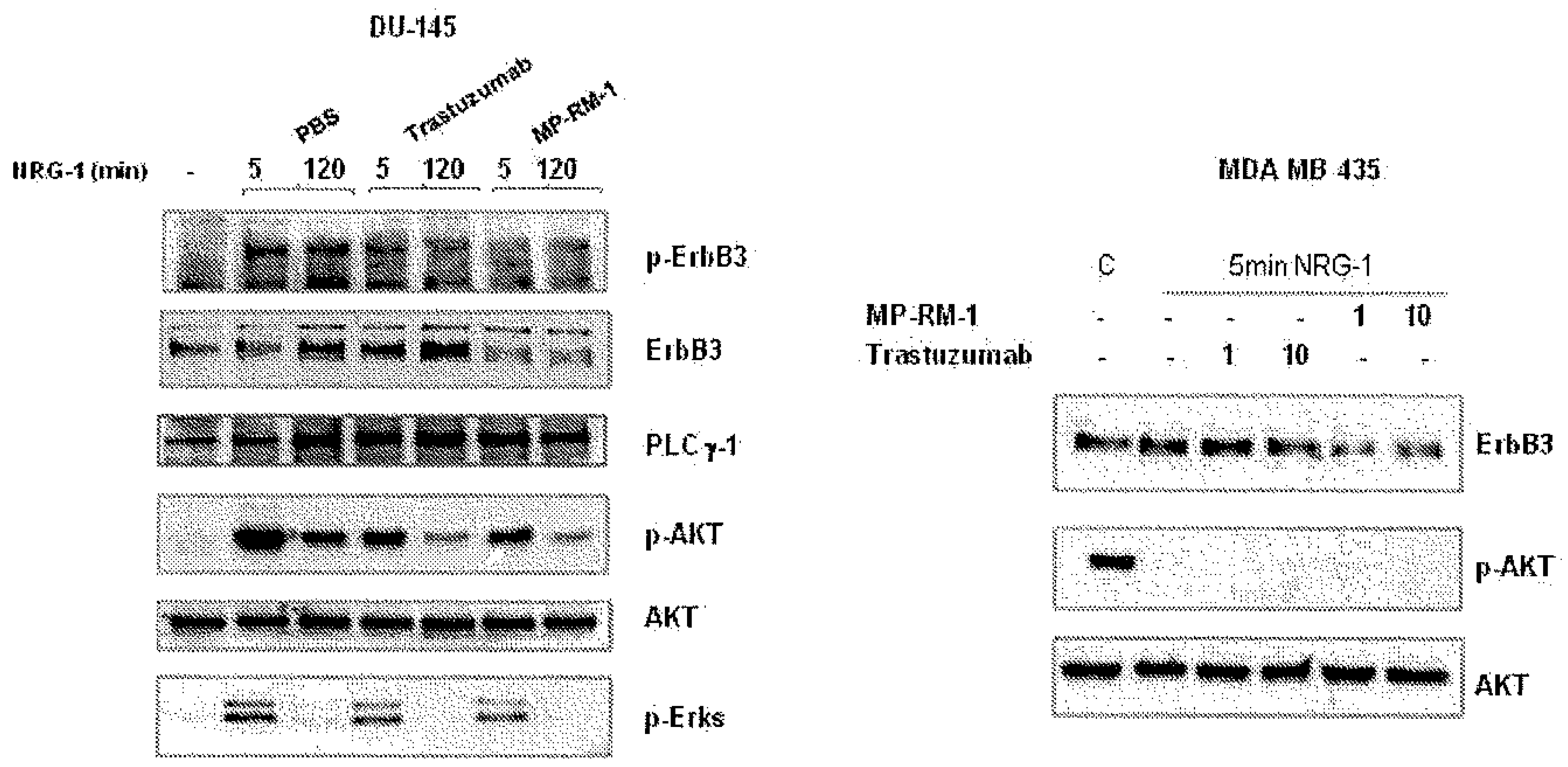

DU-145
PBS
Trastuzumab
MP-RM-1
NRG-1 (min)
-
5
120
5
120
5
120
p-ErbB3
ErbB3
PLC γ-1
p-AKT
AKT
p-Erks
MDA MB 435
C
5min NRG-1
MP-RM-1
Trastuzumab
1
10
1
10
ErbB3
p-AKT
AKT

Figure 11

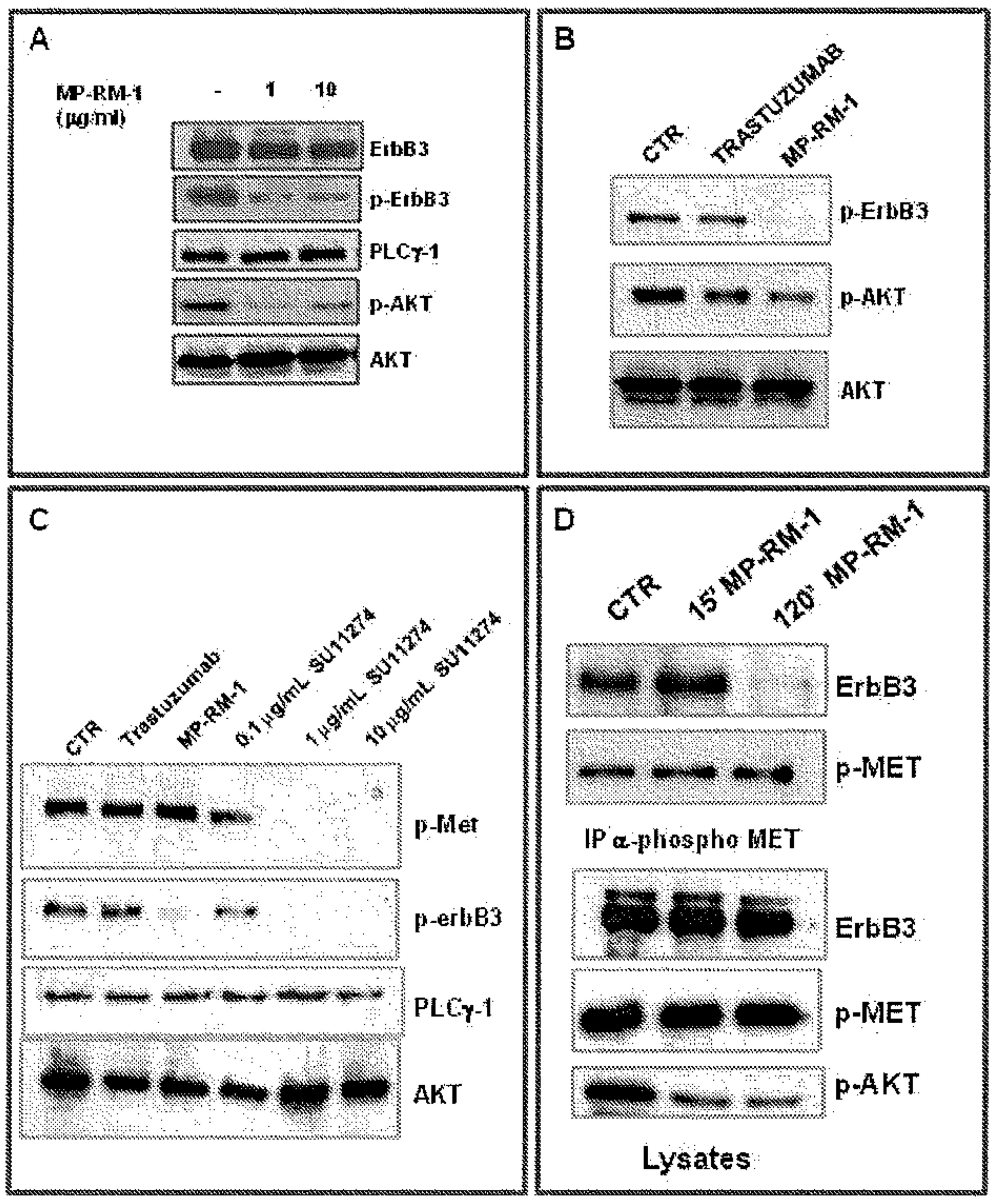

A
MP-RM-1 (µg/ml)
-
1
10
ErbB3
p-ErbB3
PLCγ-1
p-AKT
AKT
B
CTR
TRASTUZUMAB
MP-RM-1
p-ErbB3
p-AKT
AKT
C
CTR
Trastuzumab
MP-RM-1
0.1 µg/mL SU11274
1 µg/mL SU11274
10 µg/mL SU11274
p-Met
p-erbB3
PLCγ-1
AKT
D
CTR
15' MP-RM-1
120' MP-RM-1
ErbB3
p-MET
IP α-phospho MET
ErbB3
p-MET
p-AKT
Lysates

Figure 12

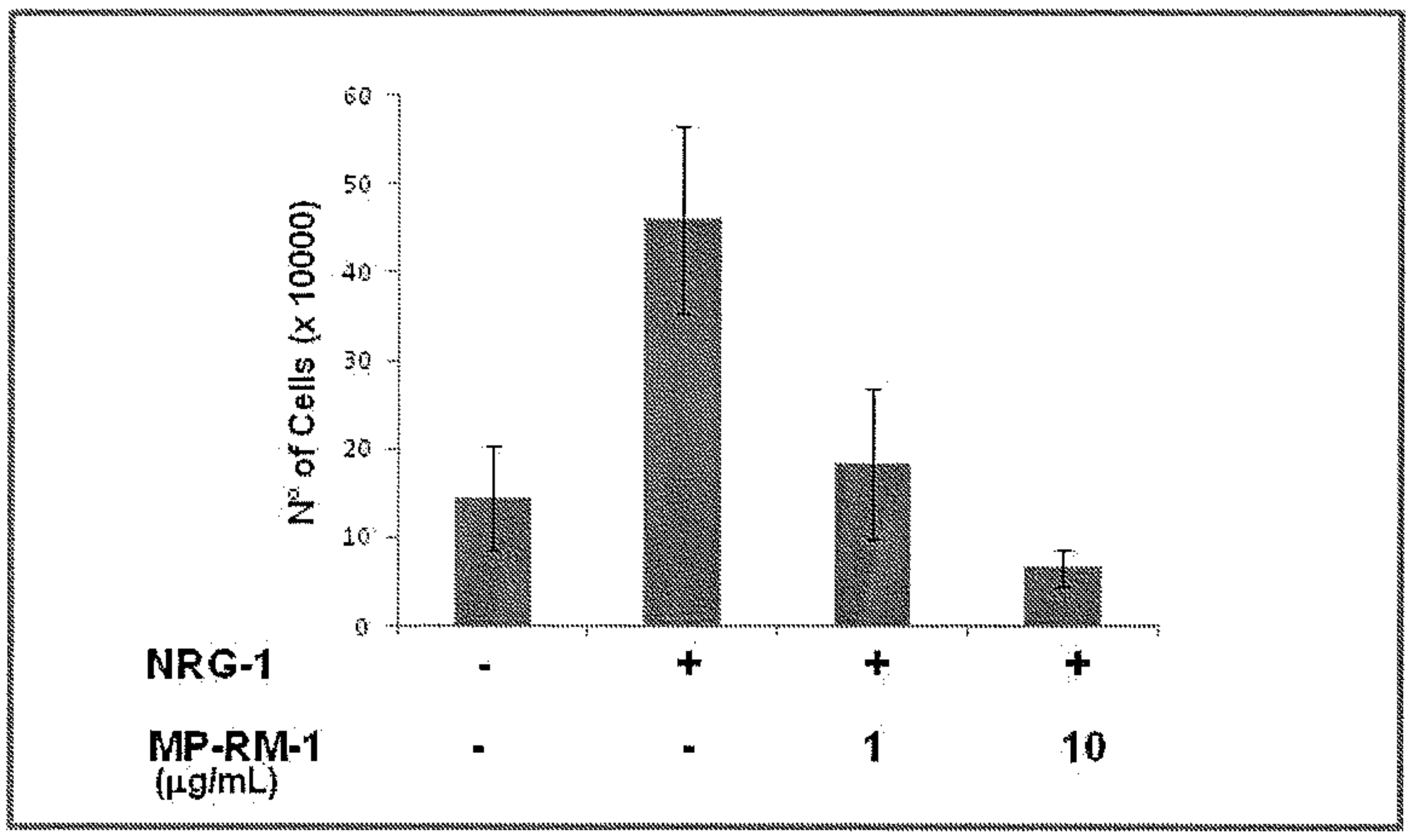


Figure 13

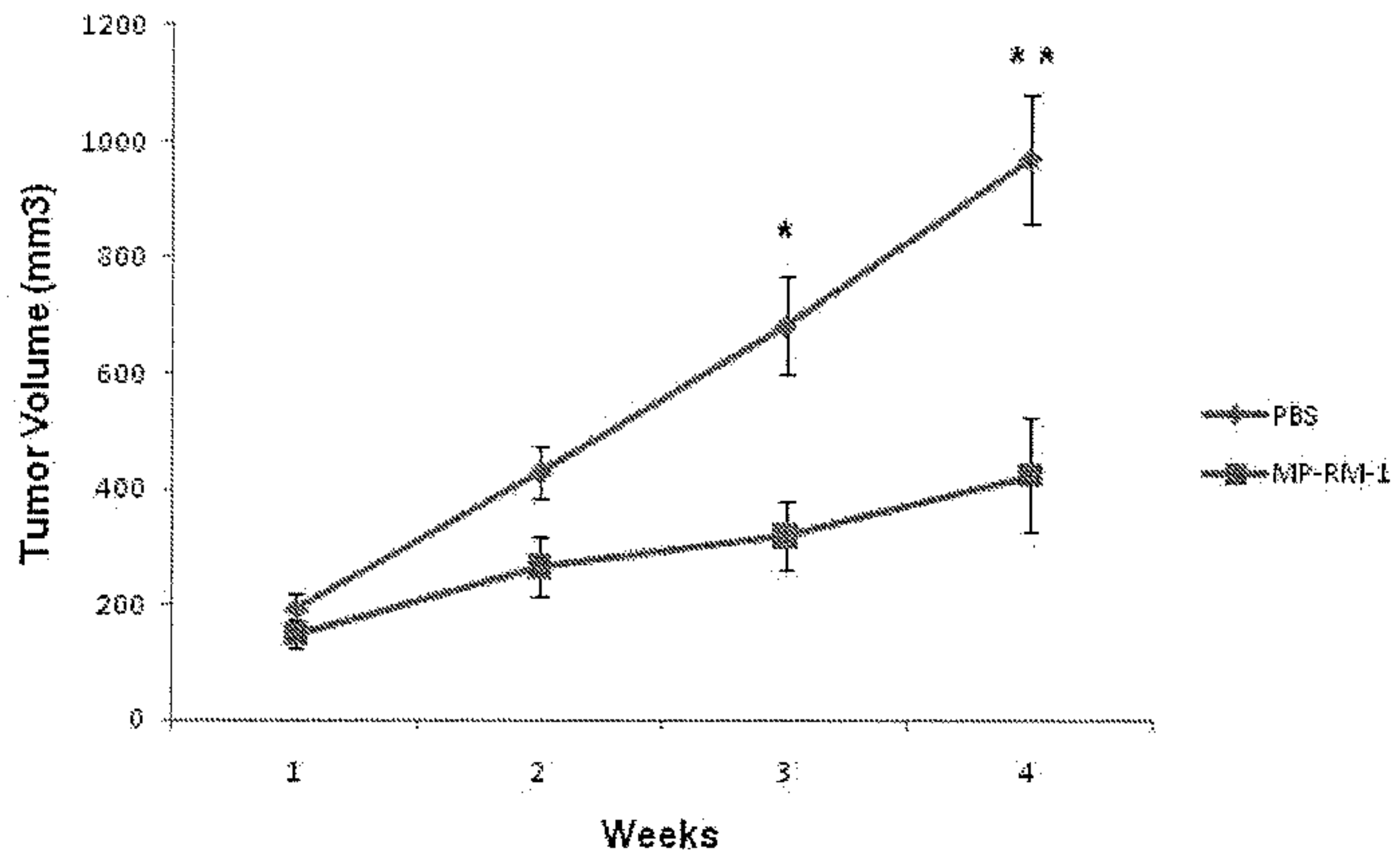

1200
1000
800
600
400
200
0
Tumor Volume (mm3)
1
2
3
4
Weeks
*
* *
PBS
MP-RM-1

Figure 14

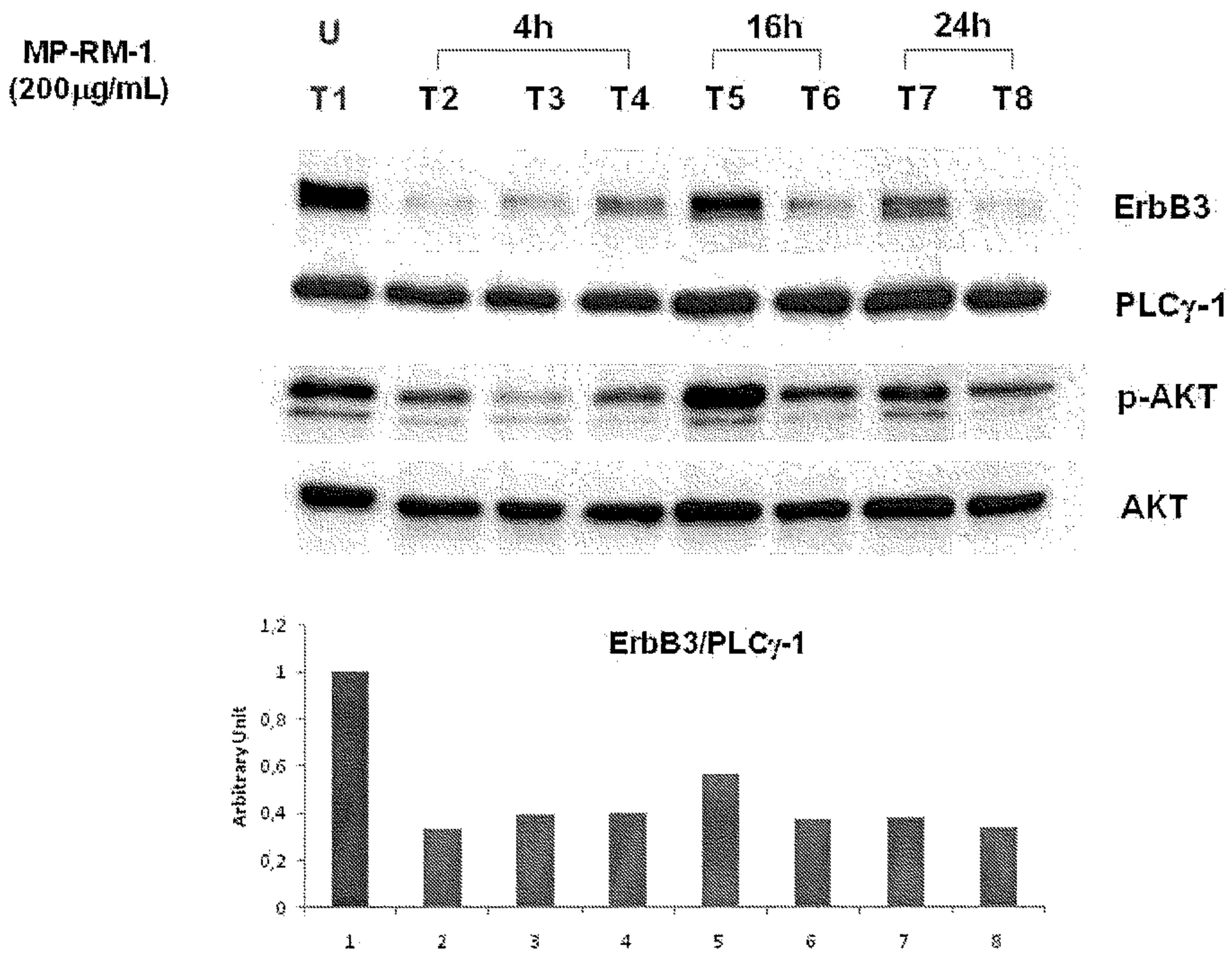

MP-RM-1
(200μg/mL)
U
4h
16h
24h
T1
T2
T3
T4
T5
T6
T7
T8
ErbB3
PLCγ-1
p-AKT
AKT
ErbB3/PLCγ-1
Arbitrary Unit
1,2
1
0,8
0,6
0,4
0,2
0
1
2
3
4
5
6
7
8

Figure 15

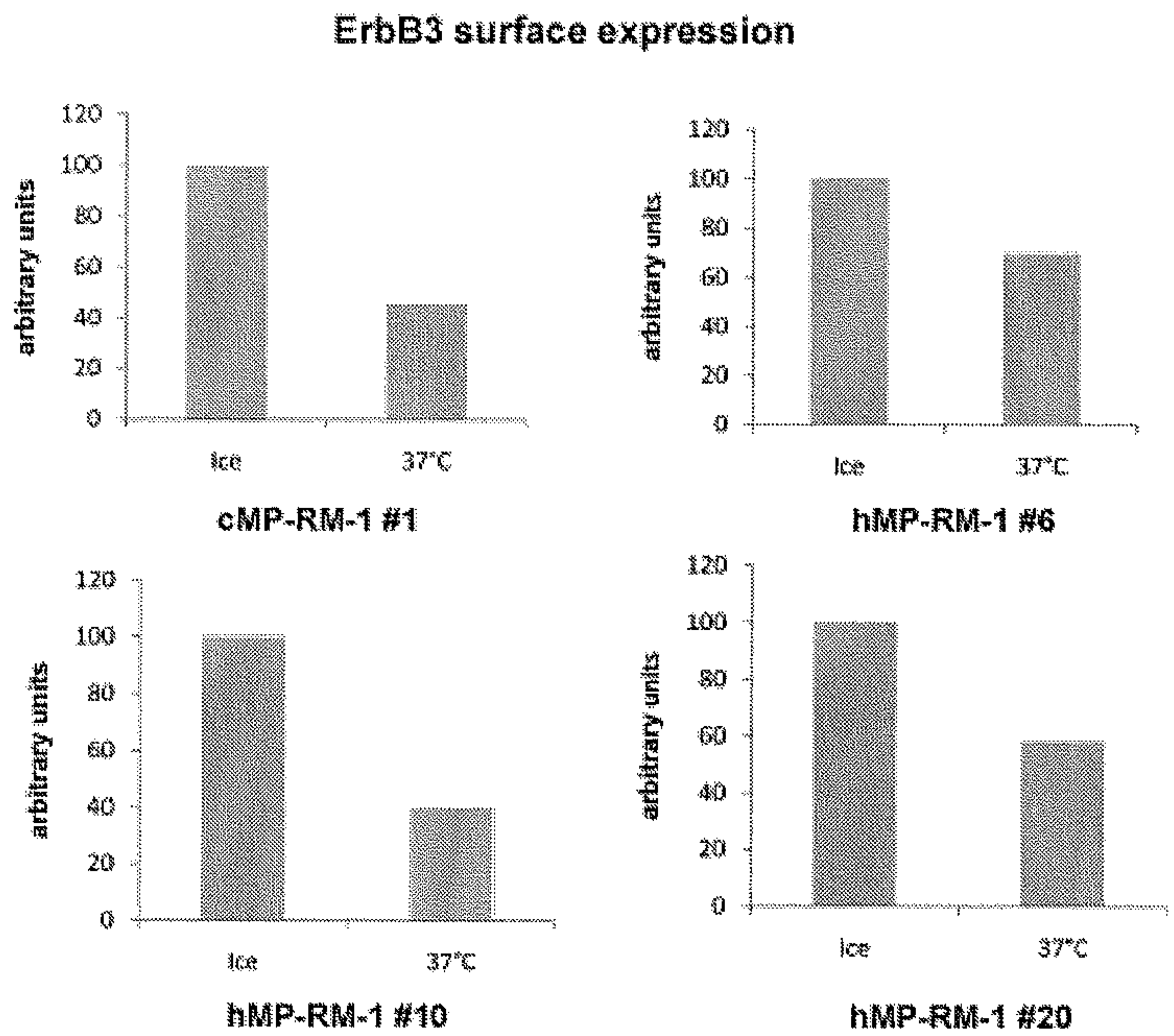

ErbB3 surface expression
arbitrary units
120
100
80
60
40
20
0
Ice
37°C
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20

Figure 16

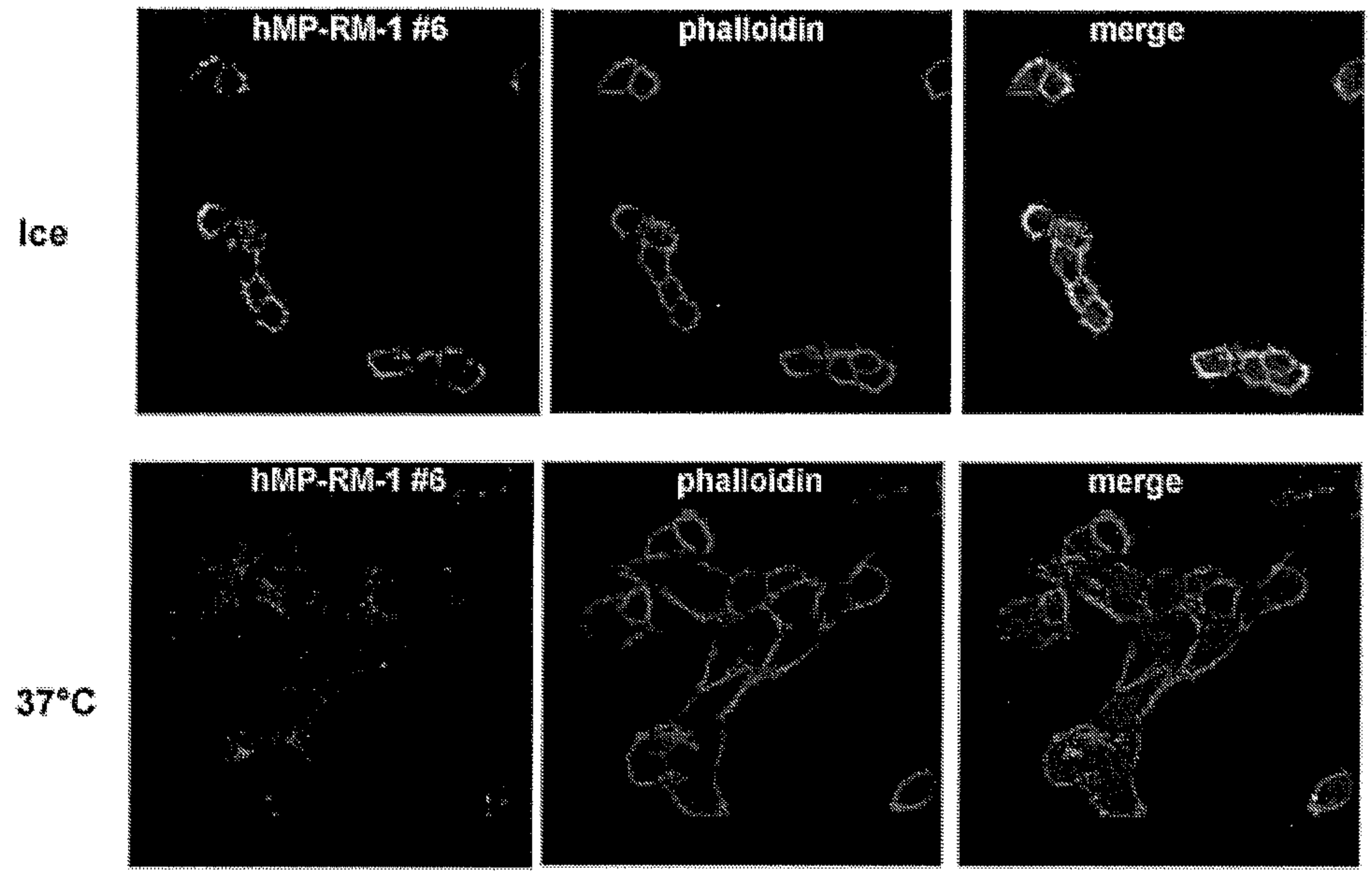

hMP-RM-1 #6
phalloidin
merge
Ice
hMP-RM-1 #6
phalloidin
merge
37°C

Figure 17

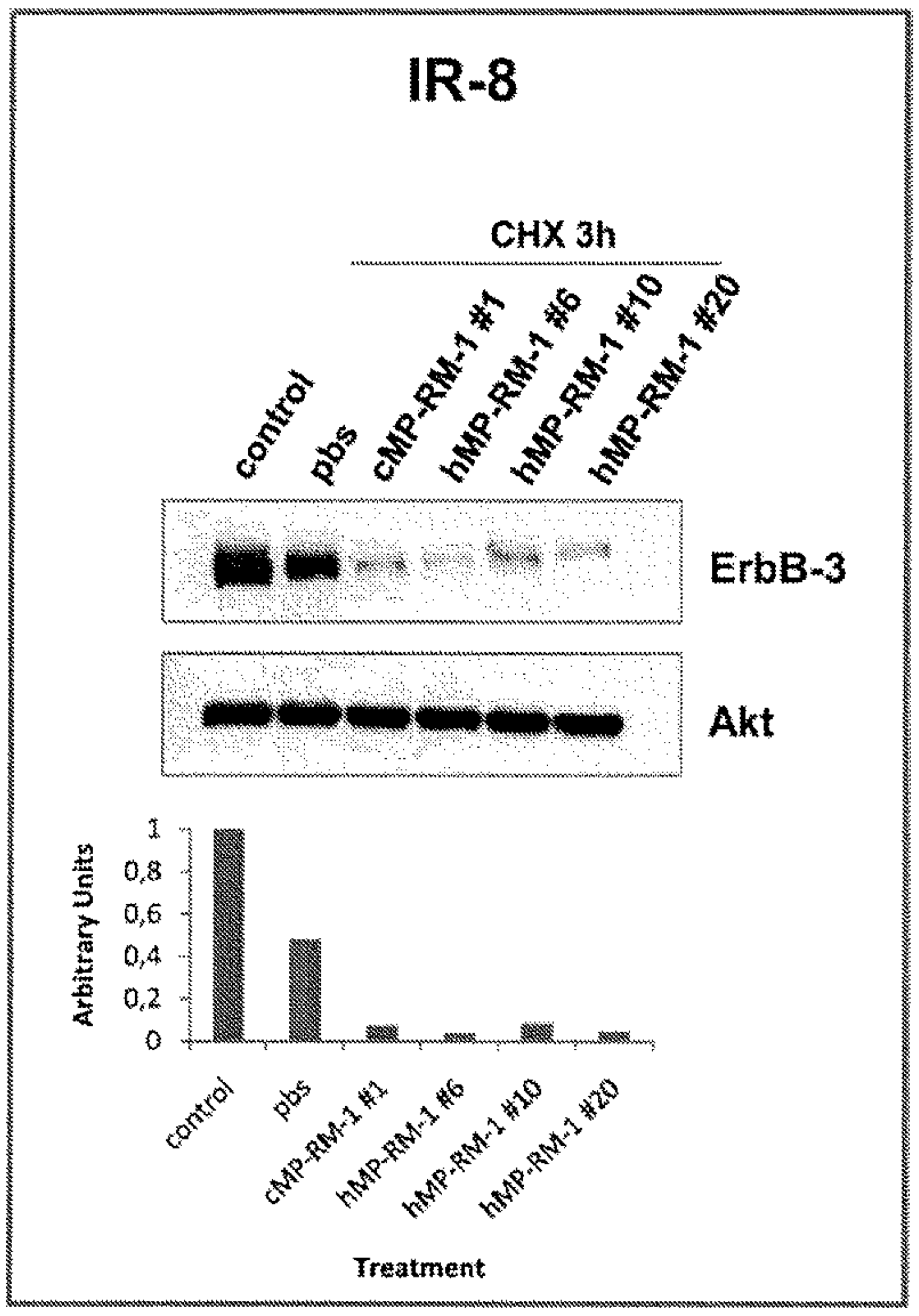

IR-8
CHX 3h
control
pbs
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20
ErbB-3
Akt
Arbitrary Units
1
0,8
0,6
0,4
0,2
0
control
pbs
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20
Treatment

Figure 18

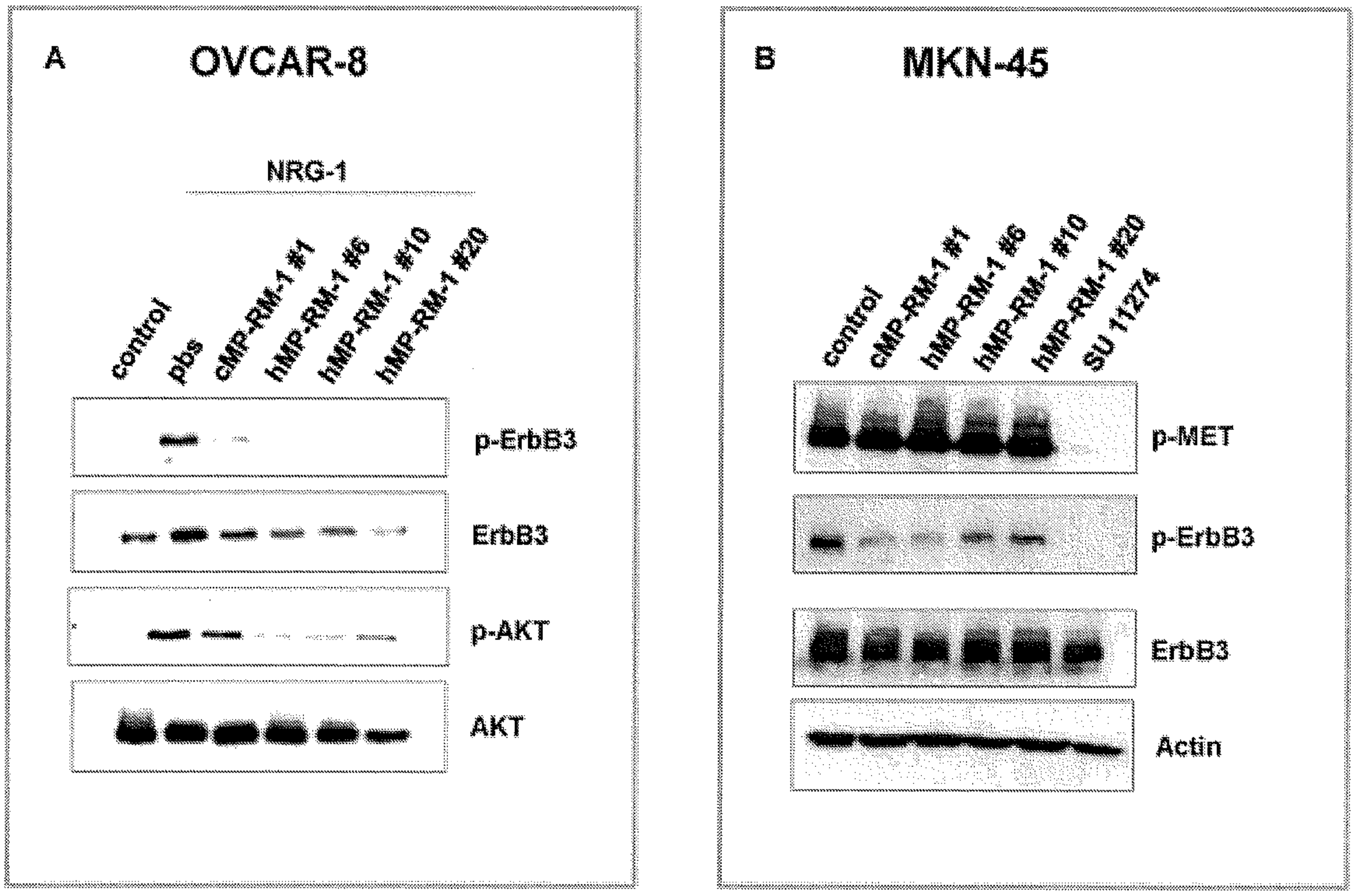

A
OVCAR-8
NRG-1
control
pbs
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20
p-ErbB3
ErbB3
p-AKT
AKT
B
MKN-45
control
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20
SU 11274
p-MET
p-ErbB3
ErbB3
Actin

Figure 19A

IR-8

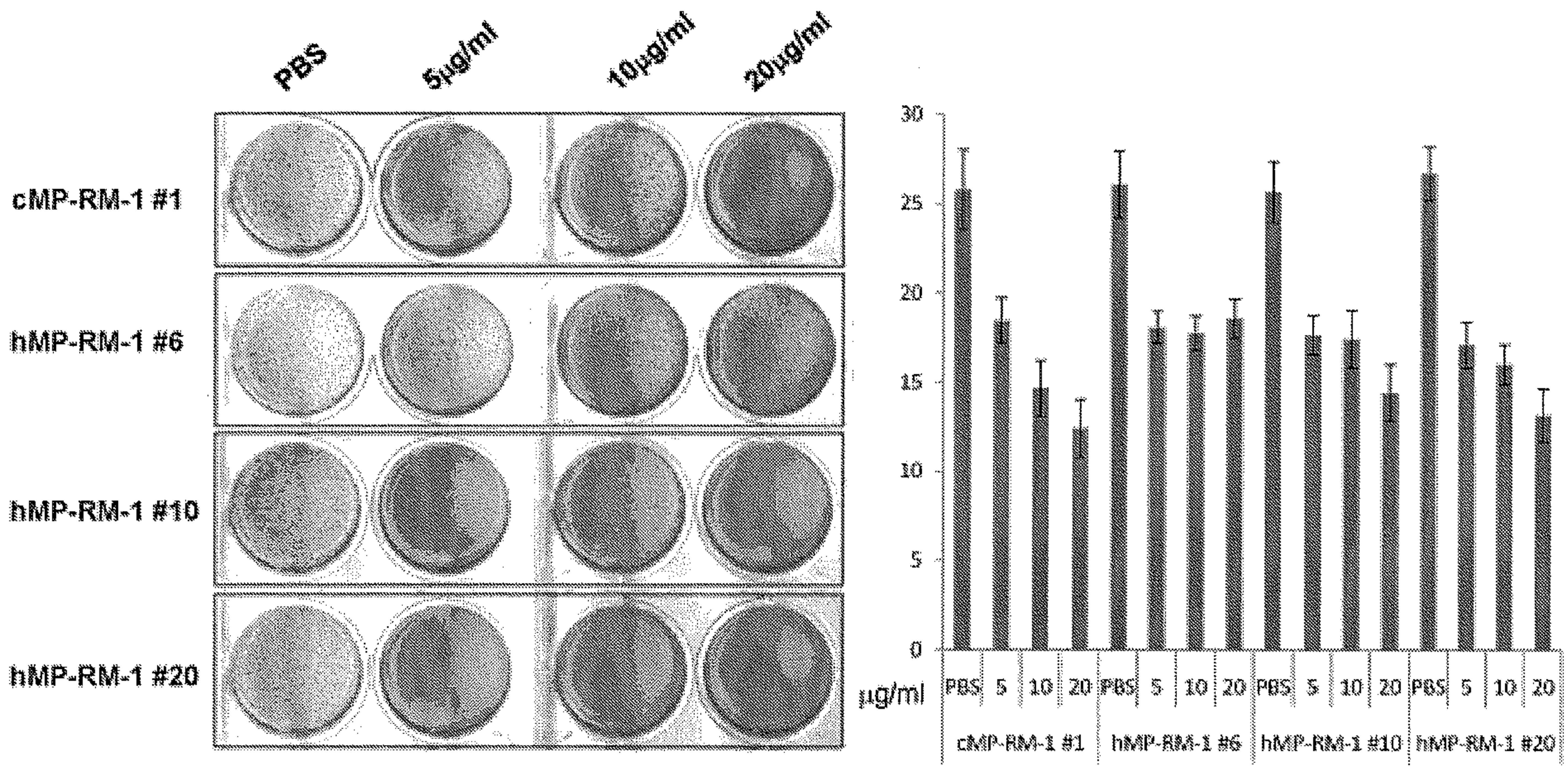


Figure 19B

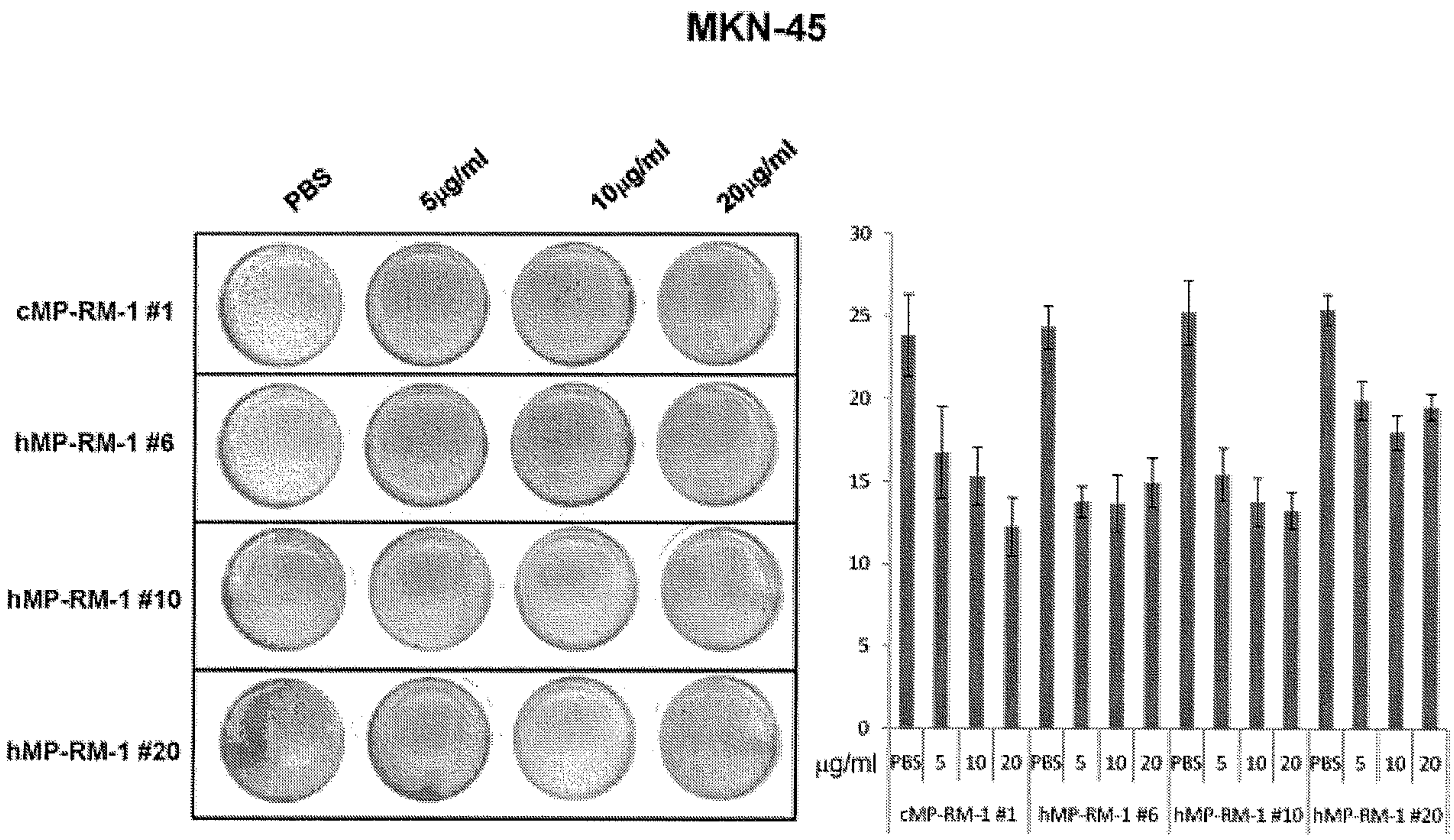
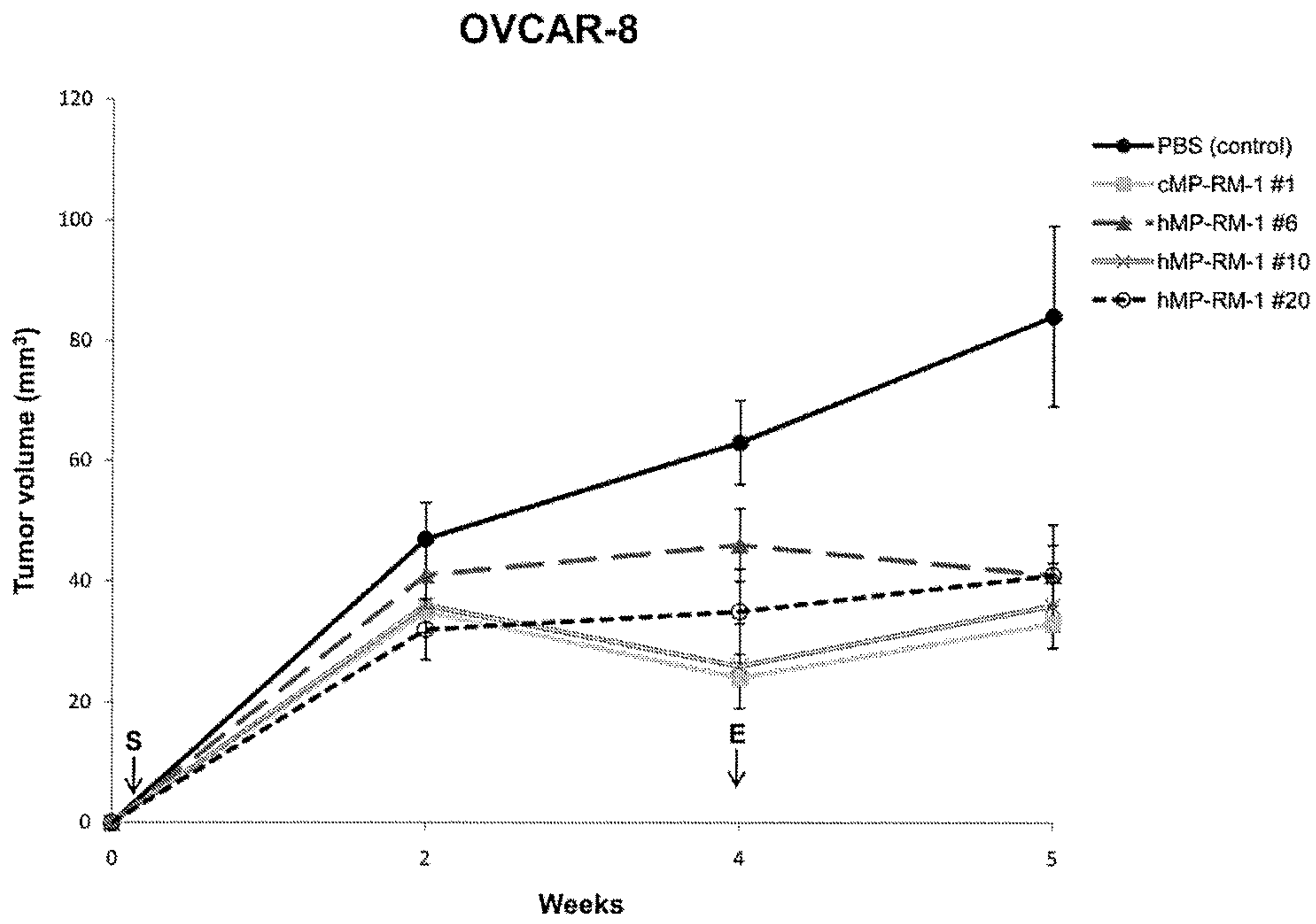
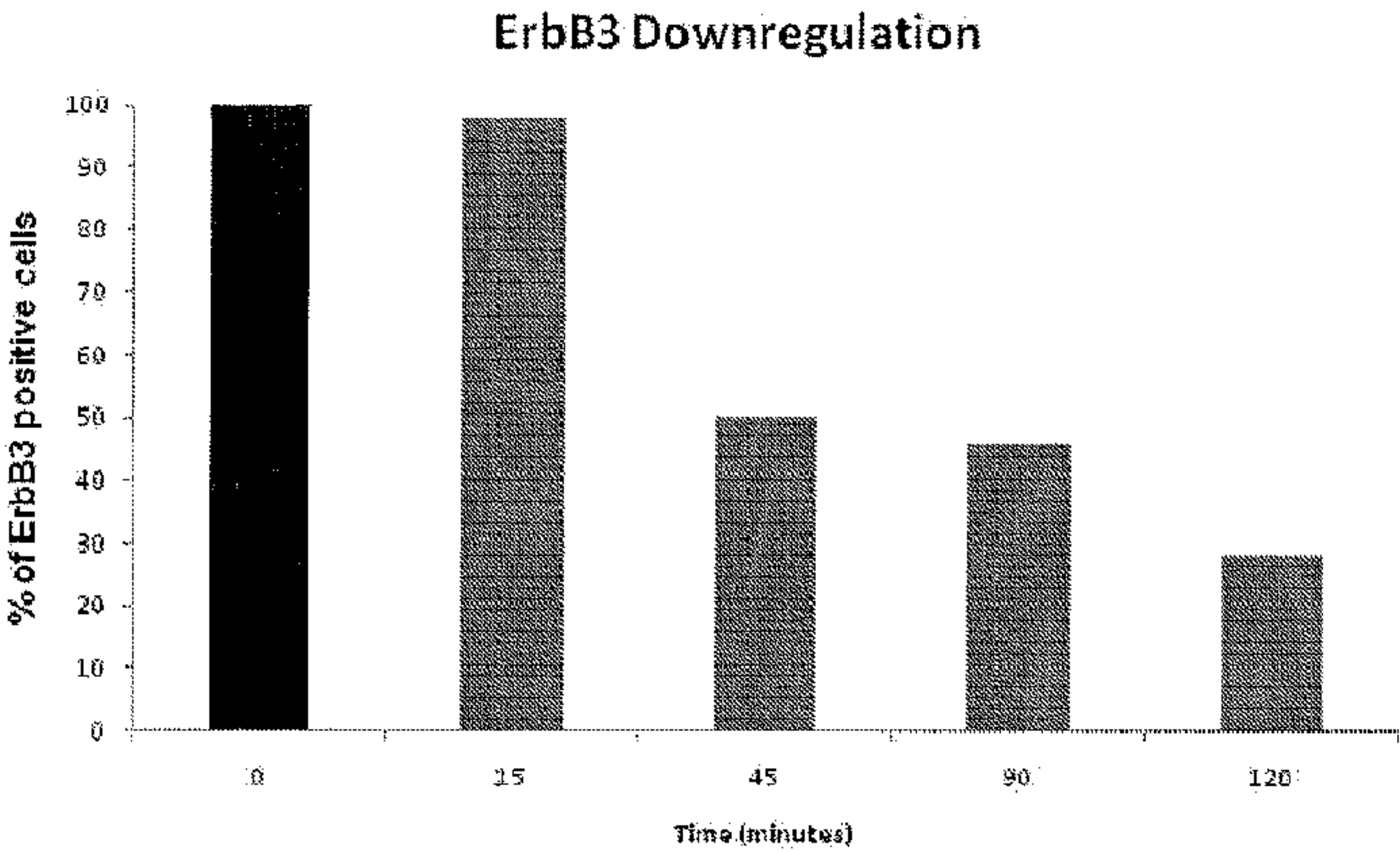

MKN-45
PBS
5µg/ml
10µg/ml
20µg/ml
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20
30
25
20
15
10
5
0
µg/ml
PBS 5 10 20 PBS 5 10 20 PBS 5 10 20 PBS 5 10 20
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20

Figure 20


OVCAR-8
Tumor volume (mm3)
Weeks
0
20
40
60
80
100
120
0
2
4
5
S
E
PBS (control)
cMP-RM-1 #1
hMP-RM-1 #6
hMP-RM-1 #10
hMP-RM-1 #20



ErbB3 Downregulation
% of ErbB3 positive cells
100
90
80
70
60
50
40
30
20
10
0
0
15
45
90
120
Time (minutes)